

UNIVERSITY
OF TASMANIA

**Investigating the effect of biochar on microbial
activities and biological processes in soil**

By

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Master of Agricultural Science

Submitted in fulfilment of the requirements for the Degree
of Doctor of Philosophy

University of Tasmania
Hobart, Australia
October, 2017

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Acknowledgements

Alhamdulillah, finally I have completed writing my thesis but of course would not have been possible without the assistance, help and support from fantastic people around me.

First and foremost, I would like to express my sincere gratitude to my supervisor Associate Professor John Bowman for the continuous support throughout my PhD study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

Also I would like to thank my co-supervisors: Dr Richard Doyle and Dr Sally Bound for their insightful comments, encouragement, enthusiasm and great efforts to accomplish this thesis which widen my research from various perspectives.

I gratefully thank Mr Stephen Paterson for assistance with the project and Dr David Ratkowsky for providing valuable advice with the data analysis. Also I would like to thank all University of Tasmania staff members and the Central Science Laboratory (University of Tasmania, Hobart) for assistance and technical support.

A special thanks to my entire extended family. Words cannot express how grateful I am to my mother and sisters for all of the sacrifices that you have made on my behalf. Your prayers for me were what sustained me thus far. I would also like to thank all of my friends and colleagues Tuflikha Putri, Ali Al-Naseri, Bianca Porteus, Colin Steedman, Peipei Zhang, Wossen Mengensha, Akhikun Nahar, Raihan Mahmud, Kaniz Mohsina, Abdelsalam Abobaker, Ala Alhusban, Shafi Ansari, Abbas Almajmaie, Hamada Esmail, Mohammed El-Astal, Syed Shah,

Tewodros Mesfin and many others for providing a stimulating and fun environment and supported me to strive towards my goal.

List of abbreviations

16S rRNA	16S ribosomal ribonucleic acid
18S rRNA	18S ribosomal ribonucleic acid
ANOVA	Analysis of Variance
AOB	Ammonia oxidizing bacteria
AWCD	Average well-colour development
BCL	Black Clay Loam
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BSL	Brown Sandy Loam
CAP	Canonical analysis of principal coordinates
cd-hit-est	Cluster Database at High Identity with Tolerance
CEC	Cation exchange capacity
CFU	Colony forming units
DAS	Diagnostic and Analytical Services
DNA	Deoxyribonucleic acid
DTPA	Diethylenetriamine Pentaacetic Acid
EC	Electrical conductivity
ESEM	Environmental Scanning electron microscopy
GHG	Greenhouse gas
L.S.D	Least significant difference
LefSe	Linear discriminant analysis Effect Size
MANOVA	Multivariate analysis of variance
MUB	Modified universal buffer

NOB	Nitrite oxidizing bacteria
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PERMANOVA	Permutation multivariate analysis of variance
QIIME	Quantitative Insights Into Microbial Ecology
RL	Red Loam
SEM	Scanning Electron Microscopy
SSU	Small subunit
TEB	Exchangeable base cations
TVC	Total Viable Count

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Abstract

Soil amendment with biochar has been widely described as a suitable approach to improve soil fertility, sequester carbon and reduce greenhouse gas (GHG) emissions to mitigate climate change. The purported benefits of biochar addition to soils include improved soil physical properties and nutrient retention as well as changes in microbial composition and abundance which in turn affect nutrient cycling in the biochar amended soils. However, the impacts of different application rates of biochar and its interactions with different soils have received less attention and need to be explored.

The aim of this thesis was to investigate the impact of biochar application rates on microbial activity and related biological processes in a range of different topsoils. This thesis focuses on understanding the behaviour of soil microbes in relating to soil biological processes that occur following biochar application and attempts to assess the relationship between these microbes and the physico-chemical properties that are altered in soil matrices after biochar application.

Field and laboratory experiments were conducted to examine the effect of biochar amended-soil on the physico-chemical and biological properties. A field experiment was conducted for 3.5 years to investigate the impact of biochar and compost amendments on soil physico-chemical properties and the total microbial community in a sandy loam apple orchard site at Mountain River in Tasmania, Australia. This was followed by a 10-month pot trial to determine the effects of biochar application rates on selected soil parameters, microbial composition and related biological processes in three topsoils. These included a reactive black clay loam (BCL), a non-reactive red loam (RL) and a brown sandy loam (BSL) topsoils. In the field experiment, soil pH

decreased in both biochar and compost treatments compared to control. However, significant differences in bacterial and fungal but not archaeal or other eukaryote community components were observed in the biochar and compost treatments. The results also indicated that biochar and compost amendments can subtly affect the community structure of the orchard soils even with active application of inorganic and organic fertilizers. There were no significant differences across a panel of enzyme activities among treatments. There were slight increases in alkaline phosphatase while fluorescein diacetate activity and hydrolysis activity slightly decreased. The overall effects on fundamental activity however are largely neutral, and likely due to the enormous structural resilience and functional redundancy present.

The 10 month pot trial showed that biochar additions had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content in both soil types and biochar loading. There was a relatively limited effect on microbial biomass in amended soils; however biochar addition reduced the potential nitrification at the higher biochar rate in the two lighter soils (RL and BSL). The addition of biochar at different loading rates was reflected in significant differences in the bacterial diversity between biochar treatments in the BSL and RL soils, while the BCL soil was more resilient to soil amendment. Complete ammonia oxidizing (*Nitrospira* spp.) and nitrite oxidizing bacteria (NOB) were more abundant than standard ammonia oxidizing bacteria (AOB) in all soils. Increased biochar loading raised the abundance of nitrifying bacteria in BCL soil while *Nitrospira* became more abundant in BSL soil. Biochar addition affected the abundance of certain N_2 -fixer groups in a soil dependent manner. Strong positive correlations were observed in *Rhizobium* ($r=0.99$) and *Azospirillum* abundance ($r=0.70$) with increased biochar loading rates in BCL. Greater biochar loading also significantly increased the relative abundance of methanotrophs, especially in BCL soil. The impact

of biochar on community structure and nitrogen cycling bacteria depended on soil type and biochar rates which correlated to the differences in soil properties. Overall, the abundance of nitrogen cycling bacterial groups seemed to be most affected by the changes in soil conditions, including aeration, C/N ratio, nutrients and pH in relation to biochar application in different soils.

Publications and conference presentation relevant to the thesis

The work presented in this thesis has so far resulted in the following peer reviewed publications.

Publications

- **Chapter 2:** Abujabhah, I.S., Bound, S.A., Doyle, R. and Bowman, J.P., 2016. Effects of biochar and compost amendments on soil physico-chemical properties and the total community within a temperate agricultural soil. *Applied Soil Ecology*, 98, pp.243-253.
- **Chapter 3:** Abujabhah, I.S., Doyle, R., Bound, S.A. and Bowman, J.P., 2016. The effect of biochar loading rates on soil fertility, soil biomass, potential nitrification, and soil community metabolic profiles in three different soils. *Journal of Soils and Sediments*, pp. 2211–2222.
- **Chapter 4:** Abujabhah, I.S., Doyle, R., Bound, S.A. and Bowman, J.P., 2017. Assessment of bacterial community composition, methanotrophic and nitrogen cycling bacteria in three soils with different biochar application rates. *Soils Sediments*, pp. 1-11. doi:10.1007/s11368-017-1733-1
- **Chapter 5:** Abujabhah, I.S., Doyle, R., Bound, S.A. and Bowman, J.P., 2016. Eukaryal community changes and composition in three different soil induced by short term biochar amendments.

Chapter 1

Literature review

1. Introduction

Biochar is increasingly being used as a soil amendment to improve soil chemical and biological properties, reduce greenhouse gas (GHG) emissions, and sequester carbon to help mitigate climate change. However, the interaction between soil microbes, soil characteristics, and the addition of biochar is not yet well understood (Lehmann et al., 2011). Recently, there is wide debate about the use of biochar and its agricultural benefits in soil. Many literature sources indicate that the application of biochar to soil influences chemical and physical properties as well as the function and structure of microbial communities in a beneficial way that collectively increases soil fertility. However, other studies have revealed that biochar addition can also have a negative impact in agricultural soils. Some biochar products may for example influence the availability and toxicity of specific elements depending on the source materials used in its manufacture (Kookana et al., 2011; Beesley et al., 2014). Different types of biochar have different impacts depending on the feedstock and pyrolysis processes used. There are a wide range of technical methods to develop biochar from a variety of materials and under different pyrolysis conditions as well. Steinbeiss et al. (2009) indicated that every technical method has a specific temperature supply and activation treatment that results in biochar with different physicochemical properties.

Biochar produced by different methods could therefore have unpredictable effects on soil functionality and fertility. Empirical studies on biochar in soil trials seem necessary

to develop a better understanding of these effects. At a basic level pyrolysis has a fundamental influence on biochar properties. In a study by Singh et al. (2010b), increased pyrolysis temperature led to increased ash content, pH, and surface basicity and decreased surface acidity. The activation treatment had by comparison little effect on most of the biochar properties. For example, wood biochars have higher total carbon, lower ash content, lower total N, P, K, S, Ca, Mg, Al, Na, and Cu contents, but lower potential cation exchange capacity (CEC) and exchangeable cations compared with manure based biochars. Sludge biochar had the highest rates of total and exchangeable Ca as well as CaCO_3 and CEC, and the lowest total and exchangeable K. Electrical conductivity (EC) values were also significantly different based on the feedstocks used to produce biochars. Wood and sludge based biochars had low EC, while manure biochars showed very high EC values (Singh et al., 2010a). These authors characterised 11 different biochars produced from five feedstocks (*Eucalyptus saligna* wood, *Eucalyptus saligna* leaves, papermill sludge, poultry litter and cow manure) at different pyrolysis conditions with and without activation, and their results indicated that biochar properties such as C content, nutrients, and the liming potential of biochars are affected by different feedstocks and pyrolysis temperature as shown in table (1.1).

Table 1.1: Mean (\pm s.e.) for pH, electrical conductivity (EC), ash content, total carbon and nitrogen contents, and CaCO_3 equivalence in 11 biochars (Singh et al., 2010a).

Biochar treatment		pH	EC (1:5) (dS/m)		Ash content		Total C (g/kg)	Total N (g/kg)	CaCO_3 * (g/kg)
			30 min	24 h	550°C	700°C			
Eucalyptus saligna wood	400°C, activated	2.67 \pm 0.10	0.13 \pm 0.01	0.44 \pm 0.01	42 \pm 4	37 \pm 2	694.0 \pm 5.1	2.1 \pm 0.2	-3.4 \pm 0.9
	550°C, activated	9.49 \pm 0.04	0.17 \pm 0.01	0.26 \pm 0.01	44 \pm 6	55 \pm 7	792.2 \pm 4.7	2.3 \pm 0.2	4.1 \pm 0.03
	400°C, non-activated	6.93 \pm 0.02	0.09 \pm 0.01	0.27 \pm 0.01	32 \pm 2	35 \pm 1	697.4 \pm 4.3	2.1 \pm 0.2	-9.2 \pm 0.04
	550°C, non-activated	8.82 \pm 0.01	0.16 \pm 0.02	0.32 \pm 0.01	44 \pm 13	33 \pm 1	836.1 \pm 7.6	2.6 \pm 0.2	7.2 \pm 1.8
Eucalyptus saligna leaves	400°C, activated	9.17 \pm 0.05	1.44 \pm 0.23	3.52 \pm 0.15	99 \pm 1	40 \pm 1	662.8 \pm 2.9	16.4 \pm 0.5	15.4 \pm 1.0
	550°C, activated	9.88 \pm 0.01	2.27 \pm 0.15	4.18 \pm 0.02	117 \pm 8	92 \pm 3	719.8 \pm 5.3	17.0 \pm 0.2	72.0 \pm 2.8
Papermill sludge poultry litter	550°C, activated	9.22 \pm 0.02	0.44 \pm 0.01	0.68 \pm 0.01	654 \pm 5	475 \pm 6	315.9 \pm 1.6	2.1 \pm 0.1	409.3 \pm 27.5
	400°C, non-activated	9.20 \pm 0.02	6.32 \pm 0.06	9.27 \pm 0.02	423 \pm 36	346 \pm 1	431.1 \pm 6.8	51.8 \pm 0.3	58.2 \pm 2.4
	550°C, activated	10.26 \pm 0.01	7.64 \pm 0.07	10.17 \pm 0.03	459 \pm 2	444 \pm 1	413.2 \pm 1.8	37.9 \pm 0.1	86.3 \pm 0.9
Cow manure	400°C, non-activated	9.03 \pm 0.01	9.18 \pm 0.14	12.28 \pm 0.19	703 \pm 4	704 \pm 7	175.0 \pm 1.5	13.5 \pm 0.1	34.8 \pm 2.1
	550°C, activated	8.94 \pm 0.02	9.64 \pm 0.08	10.97 \pm 0.12	762 \pm 6	757 \pm 5	165.3 \pm 2.5	11.4 \pm 0.2	42.7 \pm 4.1
L.s.d (p=0.05)		0.11	0.29	0.24	36	18	6.5	0.3	

*Rapid titration.

Biochar can significantly change soil physical properties, especially porosity due to the high surface area of the biochar (Kookana et al., 2011). Consequently, biochar application may influence all the aspects of soil fertility related to physical characteristics. Biochar produced from the same material might have different specific surface and porosity features depending on the pyrolysis conditions. The study by Kookana et al. (2011) showed that specific surface area and porosity were increased with increasing pyrolysis temperature; however, micropores might be destroyed at higher temperatures. The difference between biochar and the soil matrix in physical properties leads to an overall change in soil density and aggregation, hydraulic conductivity and gas transportation, which in turn affect chemical properties and microbial activity in soil (Lehmann et al., 2011).

There have been many studies that indicate biochar soil amendment enhances microbial populations and activity in soil (Kookana et al., 2011). The changes that biochar applications may cause, such as increasing total N, P, and C, exchangeable cations,

CEC etc, would be the most logical reasons for the enhancement of microbial populations and activity, however the specific changes associated with using different types of biochar still needs to be considered, especially when considering soils they are used in (Chan et al., 2008).

Greenhouse gas (GHG) emission has recently received much scientific attention due to the potential impact on climate change. This includes the release of N_2O , CH_4 and CO_2 from soil. Mitigation of climate change processes is one of the major challenges faced and many experiments have been conducted to find soil management solutions to reduce GHG. Most of the current biological and environmental studies have involved the use of biochar to suppress gas emissions and enhance carbon sequestration (Han et al., 2016; Hangs et al., 2016; Awasthi et al., 2017; Fidel et al., 2017).

Biochar applications are involved in many aspects related to soil health and quality, however, the impact on soil microbes and how they interact and adjust within biochar-modified soil environments is less understood.

2. The modification of chemical and physical properties in soil after biochar application

Biochar application as a soil conditioner has a potential effect on a range of soil properties, and the addition of biochar to soil could alter the entire agro-ecosystem depending on the physiochemical properties of the biochar. The impact of biochar application on soil physical properties including structure, texture, porosity, particle size and density, collectively may affect soil aeration, water holding capacity and microbial activity in soil (Atkinson et al., 2010). Likewise, biochar has a substantial influence on chemical properties in soil, for example, biochar addition can change the

pH, electric conductivity (EC), cation exchange capacity (CEC), and nutrient retention and availability (Gundale and DeLuca, 2007). With this overall potential to change soil systems, the understanding of the interaction between biochar and soil properties is required to begin to estimate the behaviour and impact of biochar. Detailed studies may provide the means to predict impacts of different biochar types in given soils in order to optimise benefits, be it agricultural production, carbon sequestration or GHG mitigation.

Biochar is derived from different types of feedstocks under pyrolysis conditions to produce an organic material containing high and stable organic carbon content. The physical and chemical properties of the biochar will depend on the source and feedstocks as well as the pyrolysis processes that are used to produce biochar. Spokas and Reicosky (2009) studied the impact of using different biochar with different types of soil, the results indicate that some chemical influences of biochar additions depend on both biochar properties and soil type. The diversity of biochar and its interaction with soils could have various impacts on soil properties.

2.1. Physical properties

Biochar physical properties play an important role in changing soil properties. The specific properties of biochar provide enhanced high porosity and surface area and thus potentially provide increased habitat for soil microorganisms (Fig. 1.1). Furthermore, the high CEC enhances binding of cations and anions to increase nutrient retention and availability to microbes and plants (Atkinson et al., 2010). The application of biochar could also improve irrigation management and water infiltration and enhance fertiliser treatment responses in soil. Asai et al. (2009) investigated the effect of biochar on

physical properties and rice green yields. The experiments were conducted within upland conditions at ten sites at application rates from 0-16 t/h, combined with N and P additions. The results showed an improvement in the hydraulic conductivity and there were increased rice yields in sites with low P content. There was significant synergistic response of combining biochar with fertiliser treatments. Hardie et al. (2014) also showed improved hydraulic conductivity following biochar application in an apple orchard. Improving hydraulic conductivity and other physical characteristics in soil provides suitable conditions for chemical interactions and microbial activity. Furthermore, because biochar has high resistance to microbial degradation; the impact of biochar addition in soil could persist for a long time.

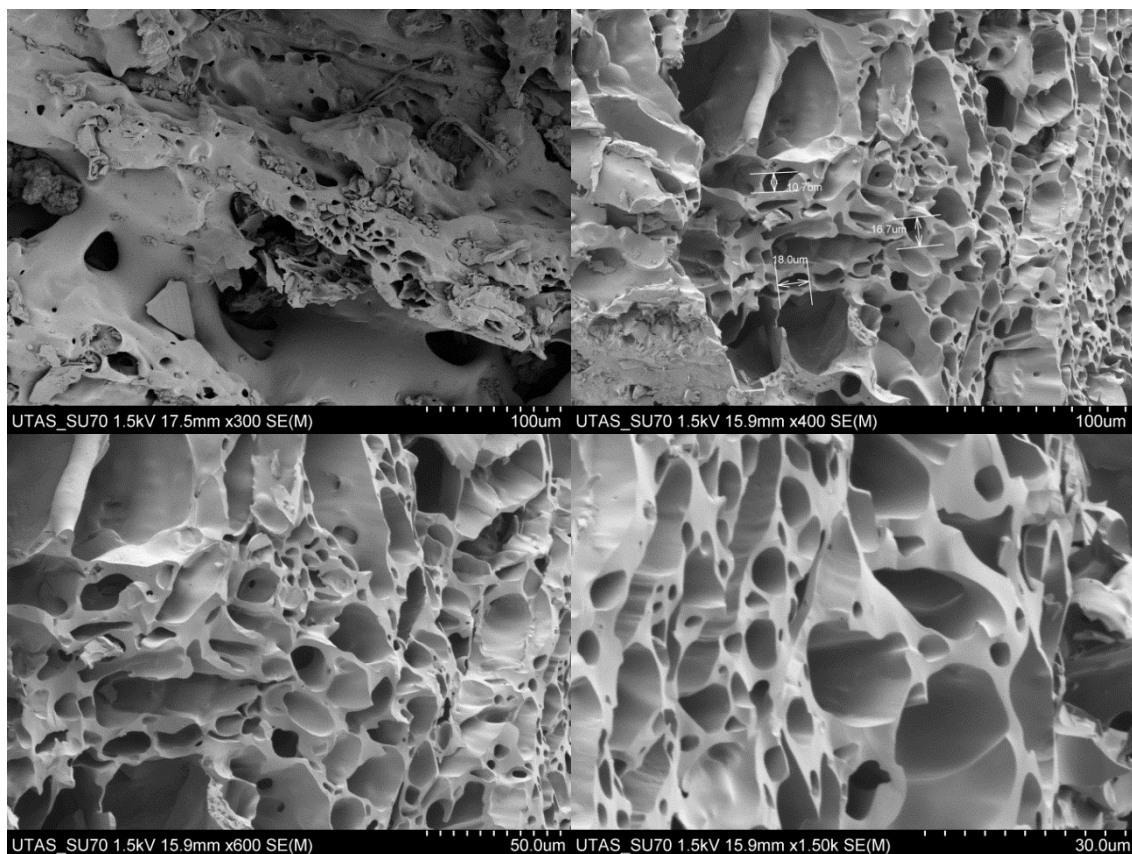


Figure 1.1: Scanning electron microscopy (SEM) images of biochar used in this study showing pore size at $\times 300$ to $\times 1500$ magnifications.

Soil physical properties are very important to soil fertility and crop production, however little is known about how this changes after incorporation of biochar. Potentially the use of biochar could be more beneficial in some soils that have poor physical characteristics, such as sandy soils. An experiment conducted by Basso et al. (2013) suggested that biochar addition increased water content in soil by around 23% compared to the control. The result also showed that bulk density of the control soil increased during the incubation time of the experiment from 1.41 to 1.45 g/cm³, while bulk density of biochar-amended soils was 9% less than the control and constantly stable during incubation time. In a study of sandy loam soil in a new apple orchard planting, Hardie et al. (2014) reported increased total porosity and saturated water content associated with a reduction in bulk density. Thus, biochar addition to sandy soil seems able to increase the soil water holding capacity, which might increase water availability in agricultural soils. Many studies have shown that biochar contributes to increased soil stability and aggregation, water management, porosity and surface area. Understanding biochar functions and effects in soil would better inform biochar choices in different agricultural soils and provide maximum benefit from using biochar as a soil amendment (Sohi et al., 2010).

2.2. Chemical properties

In the same way that biochar affects physical properties, biochar additions may alter soil chemical properties but the impacts could be more complicated. The way biochar affects soil will likely be dependent on differences in the chemical properties of biochars (Unger et al., 2011). The feedstock used to produce biochar affects specific chemical properties. For example Unger et al. (2011) conducted an incubation

experiment to determine if biochar produced under different conditions and feedstocks would differentiate the influence of biochar on soil chemical properties. In this study, selected parameters measured included total nitrogen, total organic carbon, ammonium nitrogen ($\text{NH}_4\text{-N}$) and nitrate nitrogen ($\text{NO}_3\text{-N}$). The results suggested that the reaction conditions and organic materials used to produce biochar can differentially affect specific soil chemical properties.

Biochar additions to soil can increase CEC, thus increasing nutrient holding capacity and availability of nutrients such as P, Ca, S and N. Furthermore, the increase in soil pH often observed following biochar application influences nutrient transformations and plant uptake kinetics (Fowles, 2007). It has been reported that biochar and organic fertiliser applications in soil will probably increase nutrient storage in the rhizosphere in an available form for plant roots (Steiner et al., 2007), as well as soil pH due to the liming effect of biochar (Singh et al., 2010a; Lehmann et al., 2011). Although there is much evidence of the advantages in using biochar as a soil amendment, the combination between biochar and soil types needs more investigation to understand the complexity of biochar reactions in soil.

3. The effect of biochar addition on biological processes and microbial communities in soil

The impact of biochar on biological processes and related microbes has been discussed recently by many researchers; however, there still remain some limitations, such as the complexity of agricultural soil systems, on the understanding of the interactions between biochar amended soil and biological processes, especially the direct impact of biochar on soil microbes. The main purpose of using biochar as a soil conditioner is to

reduce the expense of chemical additions, mitigate climate change-related factors (i.e. GHG) and improve overall crop production. Biochar application in soil seems to be able to achieve this partly because of its long term impact on soil systems. It is assumed that biochar can do this by altering soil biological processes such as N mineralisation and nitrification by affecting the bacteria involved in these processes through provision of a suitable environment to increase microbial activity (Berglund et al., 2004).

Biochar has been considered to be a source of highly stable carbon, which potentially affects microbial activity and nutrient cycling in soil. Due to the connection between carbon cycle and climate change, biochar has been advocated as a solution to sequester carbon, while at the same time improving soil fertility (Nguyen et al., 2008). Therefore, biochar could be a significant source of nutrients and an improved habitat for soil microbes.

3.1. Biochar and soil microorganisms

Recent studies by environmental scientists and chemists documented that biochar potentially constitutes a large percentage of the organic carbon in soil but there is still limited understanding of its impact on microorganisms and biological processes (Zimmerman, 2010). The inherent chemical and physical properties of biochar have been shown to increase nutrient retention due to the high exchangeable capacity, surface area and direct nutrient input after biochar applications (Glaser et al., 2002). However, there are many aspects relating to biochar use which are still unclear, such as the relationship between biochar and microbial functions along oxidising biochar surfaces and releasing nutrients under field conditions. Kolb et al. (2009) studied the effect of biochar addition on microbial biomass and activity, where biochar was added

to four different soils (Mollisol, Alfisol, Entisol, and Spodosol) at five application rates from 0 to 0.1 kg/kg⁻¹ biochar-soil. The results showed a significant increase in both microbial biomass and activity with increasing application rates, with the same patterns observed in all four soils although the microbial response was variable based on the available nutrient content in each soil.

Previous studies indicate that biochar may create a suitable environment for microorganisms enabling enhanced population growth and microbial abundance in soil. A variation in bacterial and fungal population ratios seems to occur because of the increases in C/N ratio after biochar addition (Kookana et al., 2011). Solaiman et al. (2010) found that P solubility increased in the presence of biochar and concluded that this was due to an increase in mycorrhizal colonisation. However, Thies and Rillig (2009) reported a decrease in microbial respiration with increasing application rates in biochar amended soil. There are conflicting results between studies, with some showing the total respiration and respiratory rate increased while mycorrhizal colonization was reduced after biochar application (Treseder, 2004; Steinbeiss et al., 2009). The differences in biochars, application rates and soil types may be contributory to various influences on the microbial community.

3.2. Biochar and microbial nitrogen transformation processes in soil

Many studies indicate that biochar applications increase nitrogen input into the agricultural ecosystem by increasing biological N₂ fixation rates as well as nitrogen availability to plants. An experiment conducted by Rondon et al. (2006) showed that biochar addition increased the amount of nitrogen fixed. Their study applied biochar at

0, 30, 60 and 90 g/kg of soil, and results indicated that the amount of nitrogen fixed into soil increased from 50 to 72% with the presence of biochar (greatest at the 90 g/kg application rate). Soil total nitrogen derived from the atmosphere was significantly increased by 49% at 30 g/kg biochar and 78% at 60 g/kg whereas this form of fixed nitrogen declined by 30% at 90 g/kg biochar levels, possibly because of low biomass production and N uptake (Rondon et al., 2006). The main reason for increased biological nitrogen fixation after biochar addition was believed to be the availability of B and Mo, while the availability of K, Ca and P, as well as increased pH and Al content status might partially contribute. The C/N ratio increased from 16 to 23.7, 28 and 35 respectively depending on the biochar rates. Since biochar seems to have a direct influence on soil microorganisms its addition may affect the activity of nitrogen fixing bacterial. Beck (1991b) demonstrated that biochar potentially affects *Rhizobium* survival in soil, observing enhanced rhizobial nodulation. Overall, several studies have demonstrated that biochar has a significant influence on the nitrogen input in soil but more studies are required to better understand the implications of long term applications of biochar on biological nitrogen fixation (Gul and Whalen, 2016; Abujabhah et al., 2017).

The form and availability of the nitrogen in soil constantly takes the attention of scientists due its great importance to soil fertility and agricultural production. The process of nitrogen transformation in soil is affected by soil characteristics, and any changes in these transformation steps, including immobilisation, mineralisation, nitrification and denitrification, will dramatically influence the nitrogen status in soil (Gul and Whalen, 2016; Wang et al., 2016). Understanding the effect of biochar addition is required to estimate both positive and negative impacts on the biological processes in the soil ecosystem.

Several studies discuss the impact of biochar on nitrogen transformation but there is limited information about the interaction between the microbial communities related to these processes and biochar in soil. The reaction of charcoal derived from fire in forest soil and the adaptation of microbial communities have been shown to influence N fixation and N transformation rates, and can immediately increase nitrogen mineralisation rates in soil (Smithwick et al., 2005). Ball et al. (2010) examined the influence of fire history in forest soil on the total and potential nitrification rates, and the nature and abundance of ammonia-oxidizing bacteria in this soil. This study showed that the relatively recent (12 year old) wildfires resulted in higher content of soil charcoal and nitrification rates compared with older wildfire events at other sites. Moreover, it has been noticed that in more recent fire affected sites there was a greater abundance of ammonia-oxidizing bacteria compared to control soils. The high abundance of ammonia-oxidizers could be the main reason for increased nitrification rates in recent wildfire sites (Ball et al., 2010). Many factors may affect the nitrification rates in soil and the nitrifying bacteria themselves; therefore, the presence of different rates and types of biochar in soil must be taken into account. For effective plant growth, adequate nitrogen must be present in soil. Biochar has been shown to increase nitrification rates (He et al., 2016b) providing nitrate (NO_3), which is the best form of nitrogen for plant uptake (Clough and Condron, 2010), biochar amendment is considered to be a suitable way to maintain the amount and availability of nitrogen in soil (López-Cano et al., 2016). Furthermore, biochar addition increases the cation exchangeable capacity thus increasing the adsorption capacity and ammonium (NH_4) storage in soil. Biochar also participates in mitigating nitrogen loss in the form of N_2O by reducing denitrification rates and improving soil aeration. Singh et al. (2010b) determined the effect of four different biochars on nitrous oxide emission and nitrate

leaching from Alfisol and Vertisol. Their results show that N₂O emission and nitrate leaching was reduced over time because of increased adsorption capacity owing to oxidative reactions on biochar surfaces as it ages. Since the concentration of the nitrogen forms in soil, such as ammonium (NH₄), nitrite (NO₂) and nitrate (NO₃) as well as the emission of nitrous oxide (N₂O), completely depends on the biological processes and activity in soil (Firestone et al., 1980), more studies are required to fully understand the biochar influence on nitrogen biological processes. Nitrogen cycle and biological transformation processes could be affected by a wide range of soil properties which could be modified after the addition of biochar to soil at different loading rates.

4. The effect of biochar additions to soil on greenhouse gas emissions (GHG) and carbon sequestration

The increase in atmospheric GHG concentrations as a result of human activity contribute to global warming, with the combination of CO₂, CH₄ and N₂O contributing to 90% of atmospheric global warming (Hansen et al., 2000). Biochar application in soil has been proposed as a global warming mitigation strategy because of its stable carbon content and the possible suppressive impact on GHG emissions from soil. Many studies indicate that charcoal applications in soil might reduce GHG emissions and suggest it as a possible and easy way to sequester carbon in soil (Aguilar-Chávez et al., 2012).

4.1. Nitrous oxide (N₂O) emissions

According to Case et al. (2012), improving soil aeration by using biochar as a soil amendment may participate in the suppression of GHG emission. This study showed

that N₂O emissions were decreased consistently following hardwood biochar amendment at 2% or more in a sandy loam soil. Improving the physical and biological immobilisation of NO₃ could be the reason for N₂O suppression (Case et al., 2012). Aguilar-Chávez et al. (2012) observed that N₂O emission declined with increasing charcoal rates during the first two weeks but no impact was observed after. Zhang et al. (2010) examined the effect of biochar amendment on N₂O emission with and without nitrogen (N) fertilisation; the biochar amendments reduced N₂O emission by 40- 51% in combination with two rates of N fertilisation, while no difference in N₂O reduction was observed between treatments in the absence of N fertiliser applications.

There is an indirect impact of charcoal applications on N₂O emission by influencing the nitrification and denitrification processes which are more likely to be affected by oxygen availability and moisture status in soil (Bremner, 1997; Clough and Condon, 2010; Bruun et al., 2011). In irrigated agricultural systems, biochar has the potential to reduce N₂O emission under different moisture conditions by enhancing soil aeration (Yang et al., 2016). Charcoal applications enhance the cation exchange capacity which increases ammonium (NH₄⁺) adsorption in soil. In other words, the adsorption of NH₄⁺ inhibits nitrogen transformations, reducing the loss of N₂O which is released during denitrification (Clough and Condon, 2010). In an aerobic incubation experiment examining the effect of rice husk biochar added into two paddy soils with and without N fertilisation, Wang et al. (2011) showed that biochar can significantly reduce N₂O emission due to the reduction of NH₄⁺-N and NO₃⁻-N content in soil. Another study conducted by Deng et al. (2016) showed that application of biochar produced at different temperatures potentially suppressed N₂O emission, however, N₂O emissions from high-temperature biochar treatments were greater than low-temperature biochar amended soils.

4.2. Methane (CH₄) emissions

Methane (CH₄) plays a significant role in the atmospheric chemistry and many studies illustrate the abundance of methane in the atmosphere is released as a result of anaerobic environment and methanogenic activity occurring in soil systems including rice cultivation, wetlands, landfills, and other agricultural practices, such as manure management (Keppler et al., 2009).

Aguilar-Chávez et al. (2012) demonstrated higher CH₄ emission in the first 20 days after biochar addition than at the end of the experiment, but there was no effect of treatment within the experiment. Another study by Zhang et al. (2010) indicated that biochar addition in the field increased total CH₄ emission because of the higher water content, however, after drainage and at low water content, CH₄ emission decreased sharply. In combinations of biochar additions and N fertilisers they reported a significantly increased impact on the total CH₄ emission with varying levels depending on the biochar amendment rates and interactions with N fertiliser, and concluded that the impact was most likely to be sensitive to the water regime within a typical rice crop management.

Decreases in CH₄ emission were reported by Rondon et al. (2005) in soil amended with biochar in both pot and field experiments. However, there is limited information about the effect of biochar applications on overall CH₄ emission from rice soil with high water content (Zhang et al., 2010). Many studies also reported that charcoal addition increased CH₄ emission from rice soil, whereas the dynamic pattern did not change significantly compared to the control (Knoblauch et al., 2008; Zhang et al., 2010).

On the other hand, Liu et al. (2011) examined the impact of biochar additions to the soil through an incubation experiment with and without rice straw. The result of this

experiment showed that both treatments reduced CH₄ emission, however, the treatment with the rice straw had a greater effect in reducing CH₄ emissions. The reason behind this reduction may be due to the suppression of methanogenic activity or the enhancement of methylotrophic activity during the incubation period (Liu et al., 2011). Therefore, using biochar derived from rice straw as a soil amendment, instead of returning the straw itself to the soil, could be an effective way to reduce CH₄ emissions. Feng et al. (2012) also stated that biochar application significantly decreased paddy CH₄ emissions which seemed to be due to the increased methanotrophic proteobacterial abundance and a decrease ratio of methanogenic to methanotrophic abundance in the biochar amended soil. Therefore, CH₄ production and consumption processes seem to be influenced by differences in moisture levels and microbial communities which may be affected by biochar application (Yu et al., 2013).

4.3. Carbon sequestration and carbon dioxide emissions

Biochar amendment is known as a suitable method to enhance carbon sequestration because of the high resistance of biochar to microbial degradation (Woolf et al., 2010). Spokas et al. (2009) confirmed that biochar application to soil is beneficial in both reducing GHG and sequestering CO₂ via mineralisation processes, but different rates of C mineralisation have been observed after biochar applications (Zimmerman et al., 2011).

Galinato et al. (2011) indicated that biochar produced from wood feedstock has 74.5 – 80% carbon content and assumed that 0.61 – 0.80 ton of carbon could be sequestered from each ton of biochar applied to the soil. Black C derived from biochar could be a significant long term approach to reduce greenhouse gas emission and enhance carbon

sequestration. Converting biomass C to biochar could capture approximately 50% of the initial carbon compared to the low amount of C normally stored in the soil after burning (3%) and during biological decomposition (< 10–20% after 5–10 years) of direct land biomass application (Lehmann et al., 2006).

On the other hand, Rogovska et al. (2011) reported that biochar applications to soil occasionally increase CO₂ emission and soil respiration rates, particularly with manure application (Rogovska et al., 2011). Yet little is known about the interactions between biochar and manure mineralization in soil in relation to C sequestration and GHG emission. Rogovska et al. (2011) estimated that biochar additions to soil considerably sequestered stable C but increased CO₂ emission rates, while the average of CO₂ emission were reduced after manure fertiliser applications. Pyrolysis processes, which are used to convert plant biomass or organic manure into biochar, could be an appropriate solution to minimise the amount of CO₂ in the atmosphere released from soil (Liu et al., 2011).

5. Thesis Approach and Objectives

This thesis focuses on understanding the behaviour of soil microbes in relation to soil biological processes that occur following biochar application. This study also attempts to determine the relationship between these microbes and the physico-chemical properties that are altered in soil matrices after biochar application.

The aim of this thesis is to more specifically investigate the use of different rates of biochars as a soil amendment and the subsequent effect on microbial activities and related processes. It is known that biochar addition has a significant impact on chemical

and physical properties which also affect the biochemical processes and microbial functions in soil. However, the relationships between microbial activity, biological processes and changes in chemical and physical properties resulting from biochar addition are still not well understood. This thesis will develop an understanding of the interactions between chemical and biological factors to devise procedures for using biochars more effectively to improve soil health and quality combined with efficient carbon sequestration and minimisation of GHG emissions.

Specific questions that were explored:

- What is the effect of different loading rates of biochar on the microbial community structure?
- Do the microbial communities in different soil types respond the same way to biochar applications?
- What effect do biochar loading rates have on the biological properties of different soil types?
- How does biochar influence nitrogen cycling in different soil types?
- How does biochar affect the nitrogen status in soil and specific microbes involved in the nitrogen biochemical cycle?

Field and laboratory experiments were conducted to examine the effect of biochar on soil physico-chemical and biological properties. Various methods were used to address these questions, including physical and chemical analysis to estimate changes in soil properties such as EC, pH, CEC, total N and P, total and exchangeable Ca, Mg, Na, and K. Molecular techniques were applied to determine the microbial community structure and their functional aspects by use of 454 and Illumina next generation sequencing.

Enzyme assays and stable isotope probing were used to measure specific biological processes, activity and efficiency of microbial components in the amended soils. Multivariate statistical calculations were applied to the data obtained during the study to discover correlations between biochar application rates, soil type and consequent effects on the native microbial population and their functionality.

Chapter 2

Effects of biochar and compost amendments on soil physico-chemical properties and the total microbial community within a temperate agricultural soil

Abstract

The use of biochar and compost as soil amendments and their comparative effects on microbial activities and related processes were investigated in an apple orchard site at Mountain River in Tasmania, Australia. Biochar derived from Acacia green waste was applied at a rate of 47 tonne ha⁻¹ just before planting and has been *in situ* for 3.5 years. Compost produced by the Luebke system was also applied separately at 10 tonne ha⁻¹ as a top dressing one week after planting. Chemical analysis indicated that there was no significant impact on total ions by either biochar or compost additions. However, organic carbon was significantly increased ($p=0.009$) by 23% for biochar and 55% for compost treatments. Soil pH decreased in both biochar and compost treatments. Microbial abundance was improved after the addition of biochar, but the effect of compost addition was greater. There were no significant differences across a panel of enzyme activities among treatments. There were slight increases in alkaline phosphatase while fluorescein diacetate activity and hydrolysis activity slightly decreased. The entire community of the soil was assessed using 16S rRNA and 18S rRNA genes amplicon pyrosequencing. Significant differences in bacterial and fungal but not archaeal or other eukaryota community components were observed. These results indicated that biochar and compost carbon amendments can subtly affect the microbial community structure of the orchard soils despite active application of inorganic and organic fertilizers. The overall effects on fundamental activity are largely

neutral, however, likely due to the enormous structural resilience and functional redundancy present.

1. Introduction

Biochar is an organic material containing a high level of carbon, and is produced by heating biomass in the absence of oxygen. It has an aromatic structure that makes it stable and highly resistant to chemical and biological degradation in soil (Atkinson et al., 2010). Biochar is increasingly being used as a soil amendment with the aim to improve soil physical, chemical and biological properties, reduce greenhouse gas emissions, and sequester carbon. Due to the specific properties of biochar, biochar addition may have significant impacts on soil chemical and physical properties, which also potentially affect the biochemical processes and microbial functions in soil. However, the interactions between biochar additions and chemical and biological properties in soil are not fully understood (Lehmann et al., 2011).

There is widespread debate about the use of biochar and its agricultural benefits in soil. Many reviews indicate that the application of biochar to soils influences chemical and physical properties as well as the function and structure of microbial communities that can be associated with an increase in soil fertility (Lehmann et al., 2011; Liu et al., 2012a; Partey et al., 2015). However, some studies have revealed that biochar addition can also have negative impacts on soil properties. Biochar can adsorb agri-chemicals such as pesticides and also organic matter which can then prevent microbial enzyme access that are subsequently released from microbial colonies (Kookana et al., 2011; Zimmerman et al., 2011). Some biochar products may be toxic depending on the source materials used in its manufacture (Kookana et al., 2011). A comparative study

conducted by Paz-Ferreiro et al. (2012) to evaluate the impact of sewage sludge derived biochar and unpyrolyzed sewage sludge on the biochemical activity on soil showed that the organic amendments had different impacts on soil biochemical activity, while the geometric mean of enzyme activities was increased in the higher biochar treatment and decreased in sewage sludge amended soil. This may indicate that pyrolyzed organic materials are suitable for enhancement of soil biochemical activity; however, impact on enzyme activity could be variable and dependent on the soil as well as enzyme (Bailey et al., 2011).

Due to its high surface area, biochar provides a habitat for soil microorganisms (Lehmann and Joseph, 2009; Kookana et al., 2011). Consequently, biochar application may influence all aspects of soil fertility related to the physical characteristics of soil. Pyrolysis conditions can influence surface area and porosity of biochar. The study by Kookana et al. (2011) showed that specific surface area and porosity were increased with increasing pyrolysis temperature; however, micropores might be destroyed at higher temperatures. The physical difference between biochar and the soil matrix leads to an overall change in soil density and aggregation, hydraulic conductivity and gas transportation, which in turn impacts chemical properties and microbial activity in soil (Lehmann et al., 2011). The application of biochar may also improve irrigation management and water infiltration and enhance fertiliser treatment response in soil. Asai et al. (2009) investigated the effect of biochar on soil physical properties and rice green yields. The results showed an improvement in the hydraulic conductivity and increased rice yields in sites with low P content and noticeable responses to the fertiliser treatments. It has been reported that compost amendment and increase soil organic content can enhance hydraulic conductivity, however the impact might be variable between different soils and application rates (Aggelides and Londra, 2000; Rawls et al.,

2003). Improving hydraulic conductivity and other physical characteristics in soil provides suitable conditions for chemical interactions and microbial activity. Furthermore, because biochar has high resistance to microbial degradation, the impact of biochar addition in soil is presumably persistent for years.

Biochar is more likely to be beneficial in soils that have poor physical characteristics, such as sandy soils. An experiment conducted by Basso et al. (2013) suggested that biochar addition to sandy soil increases water holding capacity which might increase water availability for plant use. Evidence showing the biochar contribution and its effect on soil stability and aggregation, water management, porosity and surface area indicate that understanding the biochar functions and effects in soil would assist in choosing a particular biochar in specific agricultural soils, thus gaining the maximum benefits from biochar as a soil amendment (Sohi et al., 2010).

Biochar also affects soil chemical properties but the impact could be more complex. The chemical composition of biochar differs depending on feedstocks. Unger et al. (2011) conducted an incubation experiment to determine if biochar produced under different reactions from various feedstocks would differentiate the influence of biochar on soil chemical properties, Unger et al. (2011) suggested that the reaction conditions and organic materials used to produce biochar will affect specific soil chemical properties. Biochar addition to soil can increase cation exchange capacity (CEC) and thus nutrient holding capacity, potentially resulting in increased availability of soil nutrients such as potassium (K), calcium (Ca) and nitrogen (N). Also the high cation exchangeable capacity (CEC) enhances binding cations and anions in soil to increase nutrient retention and availability to microbes and plants (Atkinson et al., 2010). Biochar has also been shown to increase soil pH, thus influencing the concentration of many nutrients in soil and their availability for crop uptake (Fowles, 2007).

The impact of biochar on biotic processes and related microbes has been discussed recently by many researchers; however, there is limited understanding of the interactions between biochar amended soil and biological processes including the direct impact of biochar on soil microbes. The main purposes of using biological fertilisers and soil amendments are to reduce the expense of chemical additions, improve crop production, and reduce greenhouse gas contributions. Biochar seems to be a beneficial way to achieve this purpose because of its long term impact on the soil ecosystem. Theoretically, biochar could alter the biological processes in soil such as N mineralisation and nitrification by affecting the bacteria which are involved in these processes as well as providing a suitable environment to increase microbial activity (Berglund et al., 2004). Several studies indicate that using biochar as a soil amendment enhances populations and activity in soil by inducing metabolism and growth of soil microorganisms (Kookana et al., 2011; Tong et al., 2014). However, the impact of biochar applications on the entire soil microbial community and how soil biota interact and adjust with carbon-amended soil environments has received little attention.

The study reported here was conducted in an apple orchard that was amended with either biochar or compost. To date the affects on soil physical characteristics (Hardie et al. 2014) and tree growth have been reported (Eyles et al. 2015). The aim of this study was to (i) understand the impact of biochar and compost on the function of soil microbes related to the biological processes that occur following application; (ii) determine the impact of these additions on the entire soil community (archaea, bacteria and eukaryotes); and (iii) determine how this relates to alterations in soil physicochemical properties.

2. Materials and methods

2.1. Site characteristics and trial design

Soil samples were collected from an established apple orchard trial site at Mountain River located in the Huon Valley in southern Tasmania (42°57'2.91"S, 147°5'52.13"E). This site was established in November 2009 during replanting of the orchard. The experimental design was a randomised complete block with four treatments and five replicates; trees were blocked on position within the tree-row. Each replicate contained three trees and plot size was 3.18 meters long and 1 meter wide. The four treatments were biochar (B), compost (C), biochar + compost (B+C) and untreated control (U); the biochar+compost treatment (B+C) was excluded and not reported in this study. Biochar was sourced from Pacific Pyrolysis, Somersby, NSW (Australia); feedstock consisted of Acacia as a whole tree green waste which had undergone pyrolysis in a continuous flow kiln at temperatures up to 550 °C for 30-40 minutes. The average pore size of the biochar, estimated by using scanning electron microscopy, ranged from 0.8 µm to 235 µm. The biochar had a pH of 6.4, contained 8.93% (w/v) organic carbon, 3 mg kg⁻¹ NH₄⁺, 1 mg kg⁻¹ NO₃⁻, extractable P of 234 mg kg⁻¹ and 1117 mg kg⁻¹ K. Physicochemical characteristics of the biochar are detailed by Hardie et al. (2014). Biochar was applied on 2nd November 2009 before tree planting, each replicate received 15 kg biochar, equivalent to 5 kg per tree space or 47 tonne ha⁻¹. The biochar was spread evenly to a width of 1 m across the tree row and worked into the top 10 cm of the soil profile. The orchard was replanted with 'Naga-Fu No 2 Fuji' trees on M26 rootstock with a 'Royal Gala' interstem. Tree spacing was 1.06 m within the row and 4.5 m between rows. The compost (produced by the Luebke system) sourced from Renew (Plenty, Tasmania, Australia) was composed of 43 % (w/v) organic carbon, 4.5 %

total nitrogen (Kjeldahl), 1.8 % water soluble nitrogen and 0.017 % nitrate nitrogen (Eyles et al., 2015). The compost was applied at 10 tonne ha⁻¹ as a top dressing within the tree row 1 week after planting on 9th November 2009. Annual fertiliser additions applied in the field site in October included N-P-K (7:3:22) at 266 kg ha⁻¹ and fresh fowl manure applied in July at 2 kg per tree. Additional nutrients in the form of calcium nitrate or potassium nitrate were supplied via fertigation from November to March at 12 kg ha⁻¹ per week, switching to Solu-K (Campbells Fertiliser Australia) in February and March. In summary, the treatments received approximately 42.5, 6.0, 131.1 and 12.0 kg ha⁻¹ per year of N, P, K and Ca, respectively. Soils were classified using the Australian Soil Classification (Isbell, 2002) as a Bleached Mottled Grey Kurosol (texture contrast) developed on Permian Mudstone with a minor contribution of Jurassic dolerite colluvium. The soil profile was described according to McDonald et al. (1998) with chemical analysis conducted by CSBP laboratories, Western Australia. The topsoil is a dark brown – black sandy loam consisting of 10.4 % clay, 72.8 % sand and 16.8 % silt. Climate data from a weather station located 7 km away indicated the site had a mean annual rainfall of 744 mm, mean maximum temperature 17.1 °C, mean minimum temperature 5.8 °C, and mean annual sunshine of 5.5 hours per day.

2.2. Sample collection and preparation

Soil samples were collected from the top 0-15 cm of the soil surface at two different times: 28th March 2013 and 17th July 2013. Samples were collected from three treatments (control, biochar, compost) and each sample divided into 4 replicates in the first sampling time and 8 replicates in the second time. All samples were placed in plastic bags, labelled and taken to the laboratory. Soil from each replicate was divided

into two parts; the first part was air dried, sieved and stored for chemical analysis and the other part stored at 4°C for biological analysis.

2.3. Chemical and physical analysis

Soil chemical analysis was conducted by CSBP laboratories, Western Australia. Properties analysed included soil water content, Colwell phosphorus, Colwell potassium, sulphur (KCl), organic carbon (Walkley-Black), nitrate nitrogen, ammonium nitrogen, electrical conductivity, pH (in 1:5 soil:water), pH (1:5 soil:0.1M CaCl₂), micronutrients by DTPA extract for copper, zinc, manganese and iron and exchangeable cations (calcium, magnesium, sodium, potassium and aluminium). Chemical and physical results were statistically analysed by ANOVA using SPSS v21 to assess the effect of biochar addition on soil properties compared to an unamended control and compost addition treatments.

2.4. Enumeration and assessment of soil biomass

Soil bacterial numbers were estimated by determining the total viable count (TVC) expressed as colony forming units (CFU) on agar plates. A modified method was conducted as described by Juhnke et al. (1987) using 10 % tryptone soy agar (Sigma-Aldrich Corp.) and incubated at 25°C for 21 days. Total biomass was estimated from extracted DNA (n=8 replicates for each treatment) which was used as an alternative method to estimate microbial biomass (Marstorp et al., 2000; Bouzaiane et al., 2007) with quantities estimated via spectrophotometer (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE, U.S.A.).

2.5. Enzyme assays

Acid and alkaline phosphatase activities in soil were assayed as described by Tabatabai and Bremner (1969) using sodium *p*-nitrophenyl phosphate salt (Sigma-Aldrich Corp.) as a substrate. Arylsulfatase activity was determined using potassium *p*-nitrophenyl sulphate (Sigma-Aldrich Corp.) as a substrate (Tabatabai and Bremner, 1970). Dehydrogenase and fluorescein diacetate hydrolytic activity were determined by colorimetric methods using iodonitrotetrazolium chloride and fluorescein diacetate (Sigma-Aldrich Corp.) respectively as substrates (Von Mersi and Schinner, 1991; Green et al., 2006). Glucosidase activity in soil was estimated using 4-nitrophenyl- β -D-glucopyranoside and modified universal buffer (MUB) (Sigma-Aldrich Corp.) as described by Eivazi and Tabatabai (1988). Amidase and urease activities were determined using methods developed by Frankenberger and Tabatabai (1980) and Tabatabai and Bremner (1972), respectively. Formamide (Sigma-Aldrich Corp.) was used as a substrate for amidase activity and urea used for urease activity assessments. An ammonia assay kit (Sigma-Aldrich Corp.) was used to determine the ammonia ($\text{NH}_4\text{-N}$) derived from amidase and urease activities.

2.6. DNA extraction and pyrosequencing

DNA was extracted from the soil samples using PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc) following the manufacturer protocol. DNA purity was measured using the spectrophotometer described previously at 260/280 nm. 16S and 18S rRNA gene tag pyrosequencing was applied to 8 to 12 replicate samples collected from the three soil plots. Tag-encoded FLX amplicon pyrosequencing of the region covered by

application of the 28F (GAGTTTGATCNTGGCTCAG) and 519R (GTNTTACNGCGGCKGCTG) primers for bacteria, 340F (CCTACGGGGYGCASCAG) and 958R (YCCGGCGTTGAMTCCAATT) for archaea, and 516F (GGA GGG CAA GTC TGG T) and 1055R (CGG CCA TGC ACC ACC) for the eukarya using a Roche 454 FLX instrument with Titanium reagents as previously detailed by Dowd et al. (2008). Approximately 3000 raw reads were obtained per sample. Sequences were denoised and chimera-filtered through a bioinformatic pipeline (Lanzén et al., 2011). Briefly, all sequences were organised by read length and de-replicated using USearch (Edgar, 2010). The seed sequence for each cluster was sorted by abundance and then clustered again with a 1% divergence cut-off to create consensus sequences for each cluster. Clusters containing only one sequence or <250 bp in length were removed. Seed sequences were again clustered at a 5% divergence level using USearch to confirm whether any additional clusters appeared. Once this process was completed any reads that failed to have a similar or exact match to seed sequences (typically poor quality reads) were removed. Chimeras were also removed from the clustered sequences created during denoising by using UCHIME in the *de novo* mode (Edgar et al., 2011). Sequences that yielded matches of <75% were discarded. CDHIT-454 (Niu et al., 2010) was used to subsequently obtain consensus clusters that were aligned via CLUSTAL-OMEGA (Sievers et al., 2011) and checked for sequence errors, chimeric sequence regions, and were taxonomically classified against the Greengenes database (McDonald et al., 2011). Potential chimeras were rechecked using Bellerophon (Huber et al., 2004). Chimeric sequences (approx. 4% incidence) were discarded. Singleton sequences were not assessed.

2.7. Clustering, ordination and diversity analysis

To assess community compositions, PRIMER6 and PERMANOVA+ (version 6.1.12 and version 1.0.2; Primer-E, Ivybridge, UK), respectively were used to conduct permutation multivariate analysis of variance (PERMANOVA) (Anderson et al., 2005), and canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003). For this analysis sequence read data organised at the lowest taxonomic level possible (usually genus to family) was normalised as percentages, square root transformed and a resemblance matrix created by calculation of Bray-Curtis coefficients. PERMANOVA was conducted using default settings with 9999 permutations, while CAP was conducted using default settings. The PERMANOVA derived significance values were considered significant when $P < 0.01$, while $0.01 < P < 0.05$ were considered only marginally significant.

3. Results

3.1. Chemical and physical properties

There was no significant direct impact on soil nutrient levels, either total or exchangeable, between treatments (Table 2.1); however, the organic carbon level was significantly different, increased by 23% ($p=0.009$) with biochar and 55% with composted treatments compared to untreated controls. Soil pH was lower in the biochar and compost treatments compared to the control (Table 2.1). The high fertiliser regime at this commercial orchard has resulted in a topsoil with high general soil fertility with moderate to high levels on N, P, K, S and Ca (see Table 2.1). The moisture content in

biochar amended soil was overall 13% higher but not significant ($p=0.319$) in relation to the control.

Table 2.1: Soil chemical characteristics of unamended control, biochar and compost amended soil, including the Least Significant Difference (L.S.D) and the p-value.

Parameters	Control	Biochar	Compost	L.S.D	p-Value
Ammonium Nitrogen (mg/Kg)	5.00	5.00	5.75	ns	0.491
Nitrate Nitrogen (mg/Kg)	112.5	81.3	114.8	ns	0.185
Phosphorus Colwell (mg/Kg)	453	393	426	ns	0.675
Potassium Colwell (mg/Kg)	494	497	507	ns	0.938
Sulphur (mg/Kg)	19.55	15.23	18.43	ns	0.323
Organic Carbon (%)	2.19^b	2.69^b	3.39^a	0.65	0.009
Electric Conductivity (dS/m)	0.29	0.23	0.28	ns	0.311
pH Level (CaCl ₂)	6.00^a	5.70^b	5.55^b	0.2708	0.015
pH Level (H ₂ O)	6.57^a	6.35^{ab}	6.15^b	0.2584	0.017
DTPA Copper (mg/Kg)	29.32	34.08	25.81	ns	0.198
DTPA Iron (mg/Kg)	128.11	153.18	154.33	ns	0.097
DTPA Manganese (mg/Kg)	4.39	5.05	5.72	ns	0.208
DTPA Zinc (mg/Kg)	13.47	14.17	16.97	ns	0.132
Exc. Aluminium (meq/100g)	0.01	0.02	0.02	ns	0.523
Exc. Calcium (meq/100g)	9.83	9.21	10.54	ns	0.434
Exc. Magnesium (meq/100g)	1.61	1.26	1.53	ns	0.118
Exc. Potassium (meq/100g)	1.02	1.09	1.11	ns	0.538
Exc. Sodium (meq/100g)	0.12	0.09	0.11	ns	0.079
Boron Hot CaCl ₂ (mg/Kg)	1.37	1.24	1.44	ns	0.582
Water Content (%)	18.07	20.41	19.99	ns	0.319

L.S.D = least significant difference, **a,b** = differences between means, **ns** = non-significant

3.2. Soil biomass

Soil bacterial numbers and total biomass was estimated by determining the TVC on agar plates (as colony forming units per gram of soil) and then by the amount of DNA extracted from the soil samples. Both the plate count and DNA concentration results showed the same patterns and positively correlated ($r=0.89$) for the biochar and compost application impact on the soil biomass. The TVC increased by 15 % in biochar amended soil (Fig.2.1A) compared with untreated soil, but compost application increased the TVC by 58 %. The amount of DNA extracted from biochar amended soil increased by 31% compared to the control whereas compost amended soil resulted in a greater increase (45%) of extracted DNA (Fig. 2.1B).

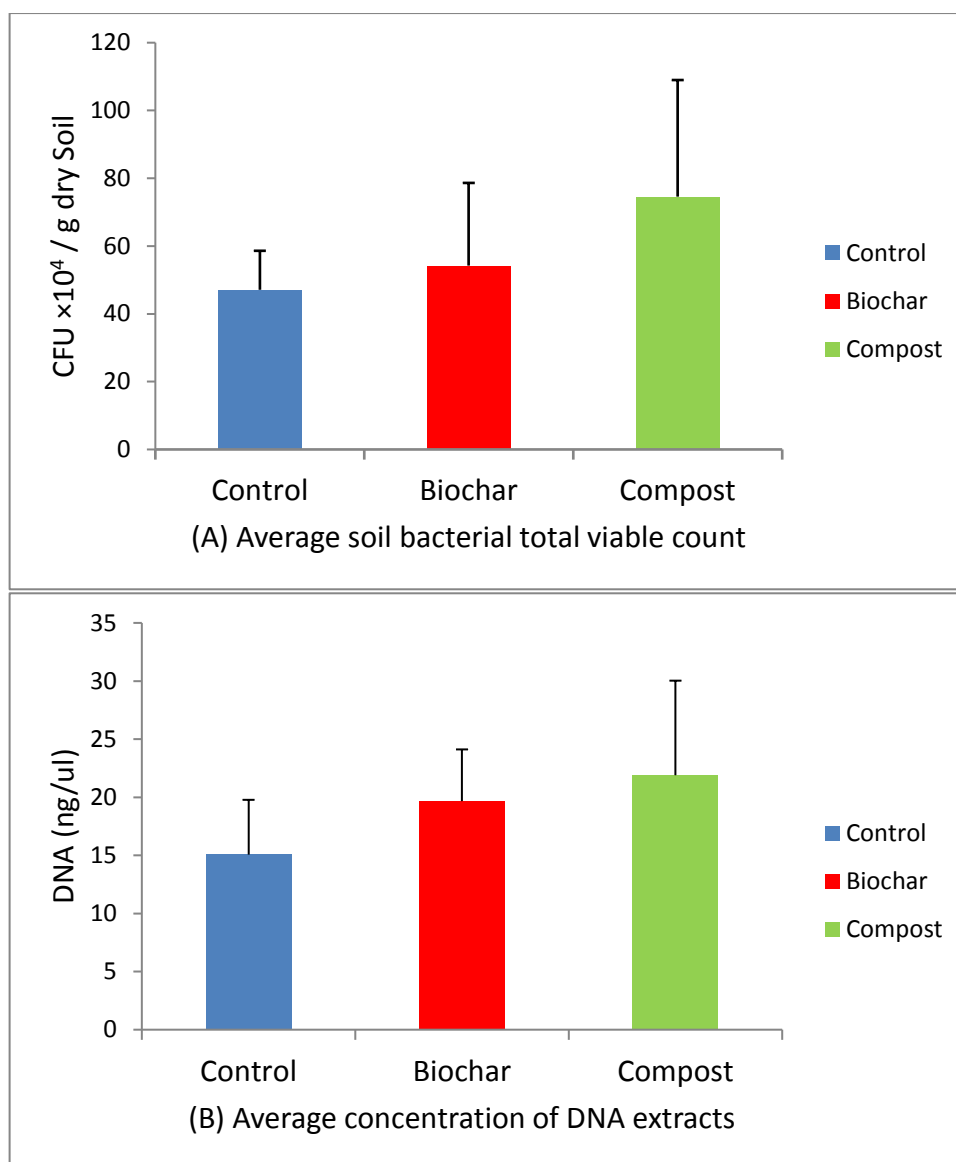


Figure 2.1: Soil total viable count (TVC) and biomass data for Mountain River control, biochar and compost amended orchard soils. (A) Average soil bacterial total viable count, estimated by determining colony number (shown as colony forming units CFU) on 10% trypticase soy agar. (B) Average concentration of DNA extracts. Error bars are the standard deviation values.

3.3. Enzyme Activity

The impact of biochar and compost application on soil enzyme activities was limited overall as indicated in Fig. 2.2. There was a significant impact on alkaline phosphatase activity ($P=0.002$). The highest activity occurred in compost plots followed by biochar treatments compared to control (Fig.2.2). There was no significant difference in the other enzyme activities among treatments except the fluorescein diacetate hydrolase activity, which was slightly decreased in the compost-amended soil.

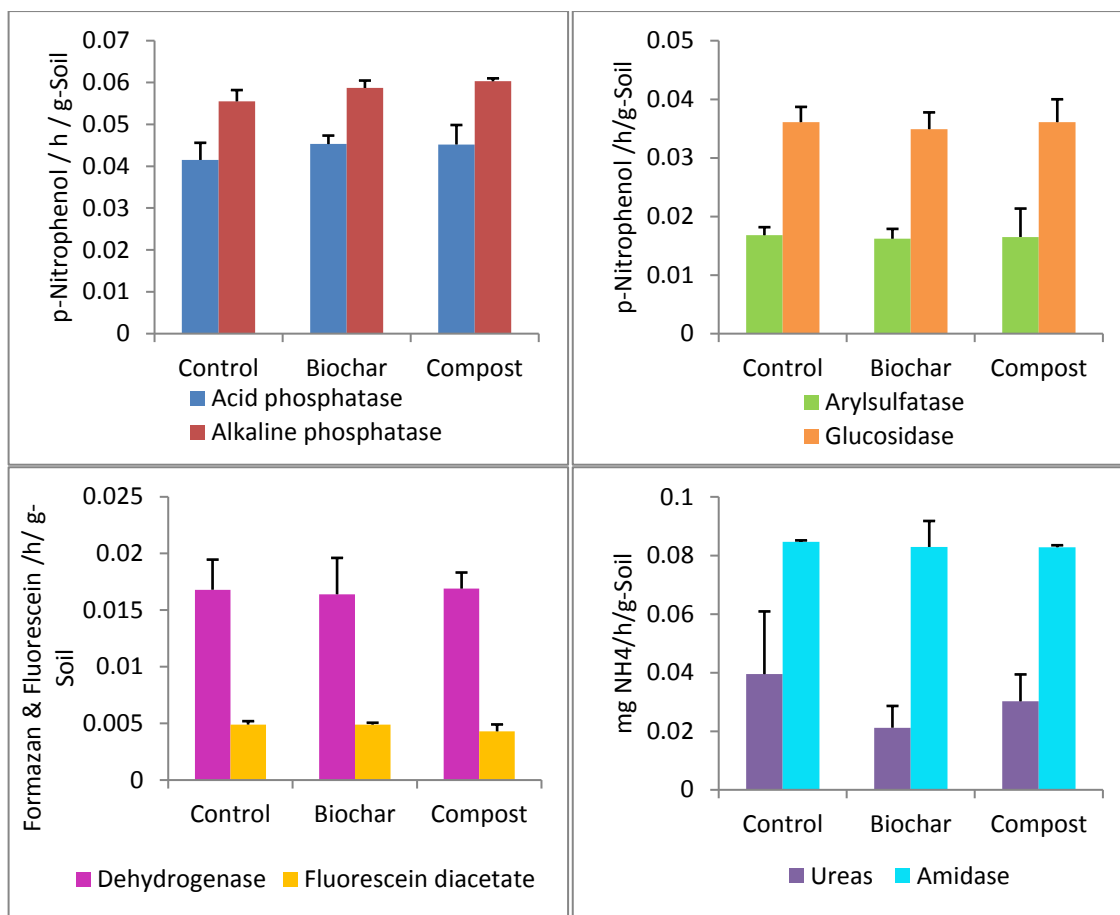


Figure 2.2: Comparison of enzyme activities between control, biochar and compost plots from Mountain River orchard soils. Error bars are the standard deviation values.

3.4. Microbial Community Structure

PERMANOVA and CAP analysis of the 16S and 18S rRNA pyrosequencing data indicated significant differences in bacterial and fungal community structures between treatments (Fig.2.3). The change in fungal community structure was highly significant ($p=0.0004$) whereas fewer differences were observed in the bacterial community structure ($p=0.0109$) between unamended control, biochar and compost amended soils as shown in Fig.2.4.

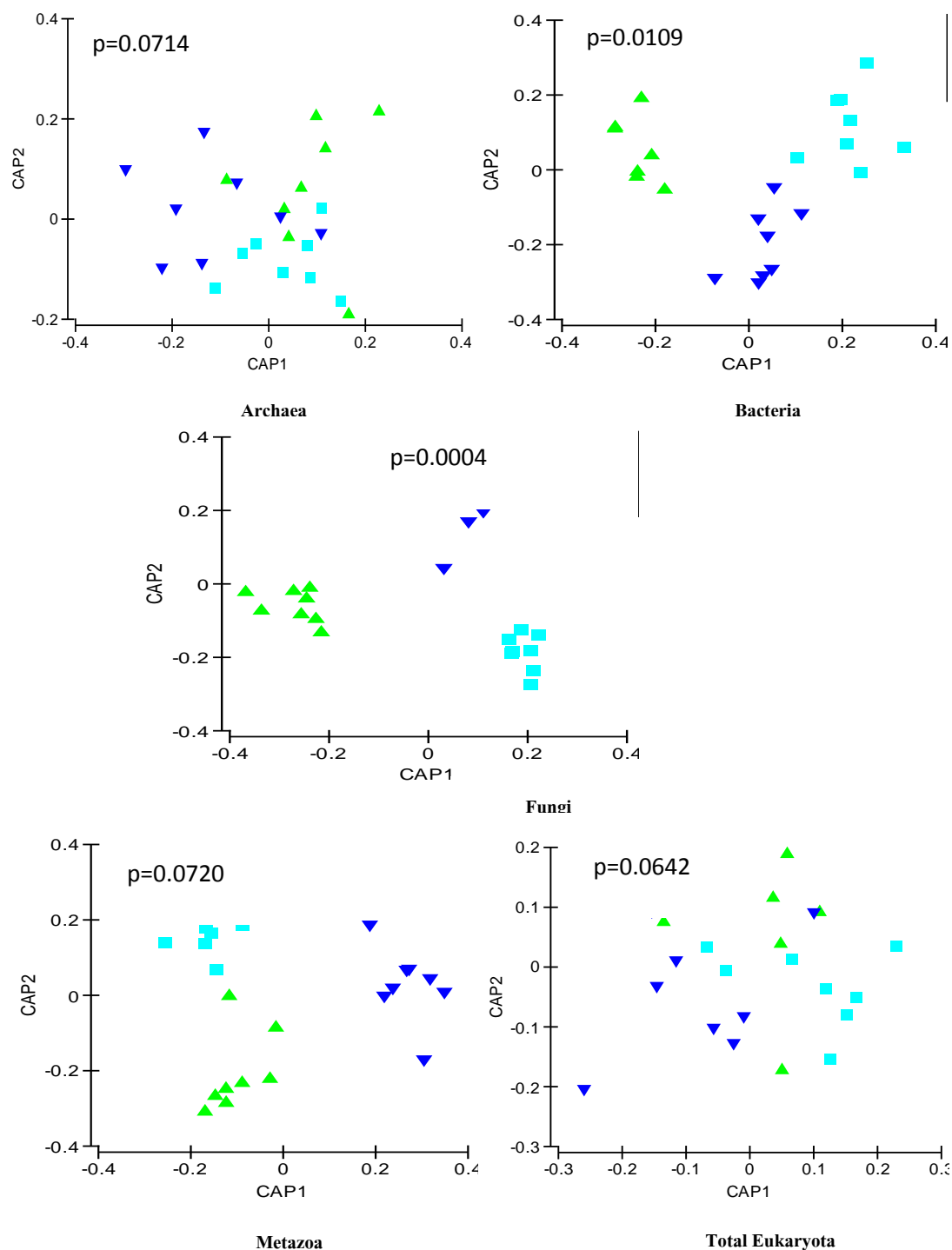


Figure 2.3: Canonical analysis of principal coordinate (CAP) plots of microbial and eukaryotic community structure determined from taxa classifications derived from 16S and 18S rRNA gene sequence analysis data. Comparisons are shown between unamended control, biochar and compost-amended soils. The respective treatment symbols are: ▲ control, ▼ biochar, and ■ compost. Each symbol represents an individual soil sample. The classification of replicate data treatment for each of the community components was assessed using PERMANOVA. Significance values for this assessment are shown for five major community components.

3.4.1. Effect of carbon amendments on *Archaea*

Soil archaeal communities, made up nearly completely of ammonia oxidizers of class *Nitrososphaerales* (phylum *Thaumarchaeota*) did not show any statistical changes in structure between amended and control soils (Fig. 2.3). It however should be pointed out that the contribution of archaea to the different soils relative to bacteria and eukarya was not measured in this study.

3.4.2. Effect of carbon amendments on *Bacteria*

The dominant bacterial group within treatments at the phylum level was *Proteobacteria*, which had proportions of 38%, 41% and 46% in control, biochar and compost treatments, respectively. At the class level (Fig. 2.4), *Alphaproteobacteria* increased by 12% in the biochar and 47% in the compost treatment compared with the untreated control, followed by *Betaproteobacteria* which increased by 11% in biochar and 7% in compost treatments. *Gammaproteobacteria* increased by 10% in both biochar and compost treatments while *Deltaproteobacteria* increased by 10% and 16% in biochar and compost treatments compared to the control. *Flavobacteriia* decreased by 34% in biochar and 70% in compost treatment compared to control. *Acidobacteriia* decreased by 5% in both biochar and compost treatments. Likewise, the proportions of *Sphingobacteriia* and *Gemmatimonadia* decreased, specially in compost treatments (Fig. 2.4). However, *Rubrobacteridae* increased by 30% in biochar and 48% in compost compared to control. No significant changes were observed in class *Nitrospira* in biochar treatments, while *Nitrospira* increased by 54% in compost treatments.

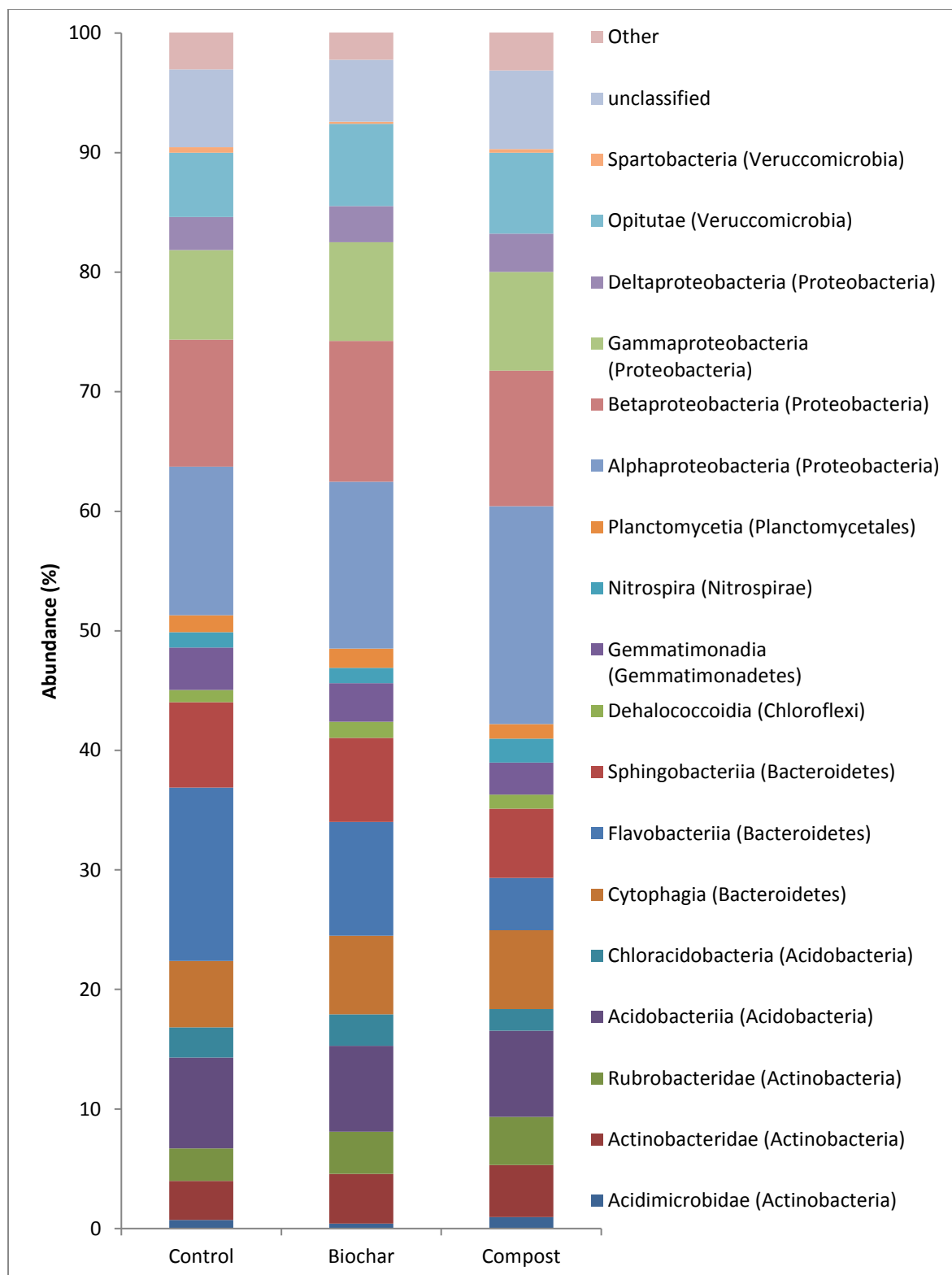


Figure 2.4: Averaged proportions of bacterial taxa at the class level in the control, biochar and compost-amended orchards soils identified using 16S rRNA gene amplicon sequence analysis.

3.4.3. Effect of carbon amendments on *Fungi*

The impact of biochar and compost addition ($p=0.0004$) varied between fungal groups (Table 2.2), some fungal groups became relatively less abundant in the treatments compared to the control, including *Entomophthoromycota*, *Chytridiomycota* and *Basidiomycota*, whereas the abundance of *Ascomycota*, *Blastocladiomycota* and *Glomeromycota* was greater in either the biochar or compost treatments. The *Ascomycota* increased by 39% in biochar and 48% in compost compared to control, while the *Glomeromycota* (arbuscular mycorrhiza) significantly increased in the compost compared to control and biochar treatments (Table 2.2). The impact of biochar and compost additions was greatest on ascomycetes of class *Pezizomycetes* (apothecial fungi), which increased by 149% in biochar and 190% in compost compared to the unamended control. Sordariomycetes (fungi with perithecial fruiting bodies), which was the most abundant fungal group overall, also increased by 31% and 41% in biochar and compost treatments (Fig. 2.6).

Table 2.2: The mean proportion of reads of fungal phyla in an untreated control, biochar and compost-amended orchard soil.

Fungal phylum	Relative Abundance (% of reads)		
	Control	Biochar	Compost
<i>Ascomycota</i>	51.1	71.0	75.5
<i>Basidiomycota</i>	33.9	10.7	10.2
<i>Chytridiomycota</i>	5.6	5.0	3.9
<i>Entomophthoromycota</i>	0.5	0.3	0.3
<i>Blastocladiomycota</i>	0.3	0.3	0.3
<i>Mortierellales</i>	0.05	0.05	0.01
<i>Glomeromycota</i>	0.03	0.05	0.6
<i>Unclassified fungi</i>	8.5	12.7	9.2

3.4.4. Effect of carbon amendments on *Metazoa* and other *Eukarya*

CAP analysis (Fig. 2.3) showed a slight change in the proportions of soil metazoa in the biochar and compost treatments compared to the unamended control ($p=0.072$). The abundance of the dominant soil metazoa is shown in Fig. 2.5. Addition of biochar resulted in an apparent increase in soil nematodes compared to control and compost treatments. The proportion of nematodes of class *Chromadore* barely changed in biochar but decreased in compost treatments by 23%, while nematodes of the class *Enoplea* increased by 14% and 25% in biochar and compost treatments respectively. The relative proportion of reads of *Annelida* was in general reduced in biochar and compost treated soil. Arthropods in the class *Arachnida* were 28% less abundant in the biochar treatment but 23% higher in the compost treatment compared to the control, while the abundance of *Ellipura*, which includes proturan and collembolan arthropods, was increased in both biochar and compost by 421% and 346% compared to control; *Chilopoda* (millipedes and relatives) were most abundant in the compost treatment, approximately 10% of the total Metazoa in compost amended soil.

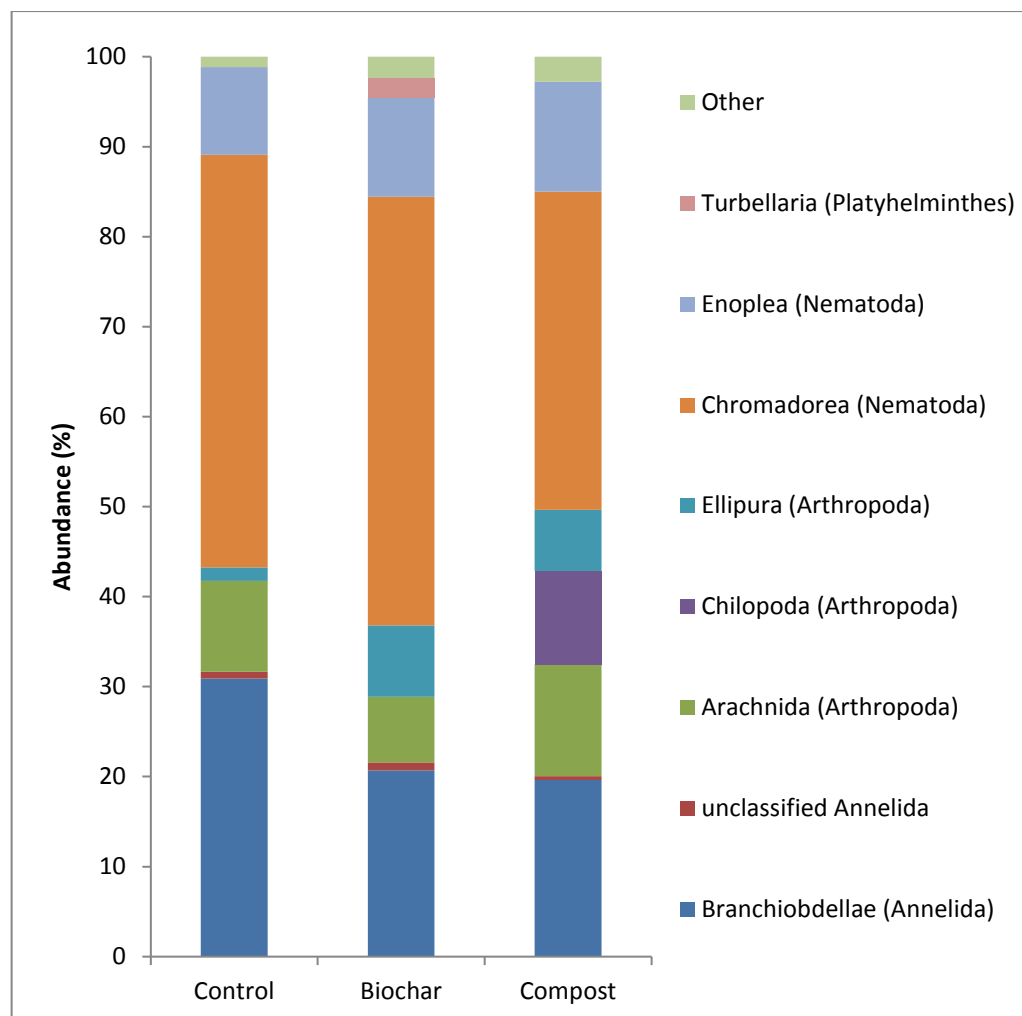


Figure 2.5: Averaged proportions of dominant soil metazoa at the class level as determined from 18S rRNA gene amplicon sequence analysis in the control, biochar and compost-amended orchard soils.

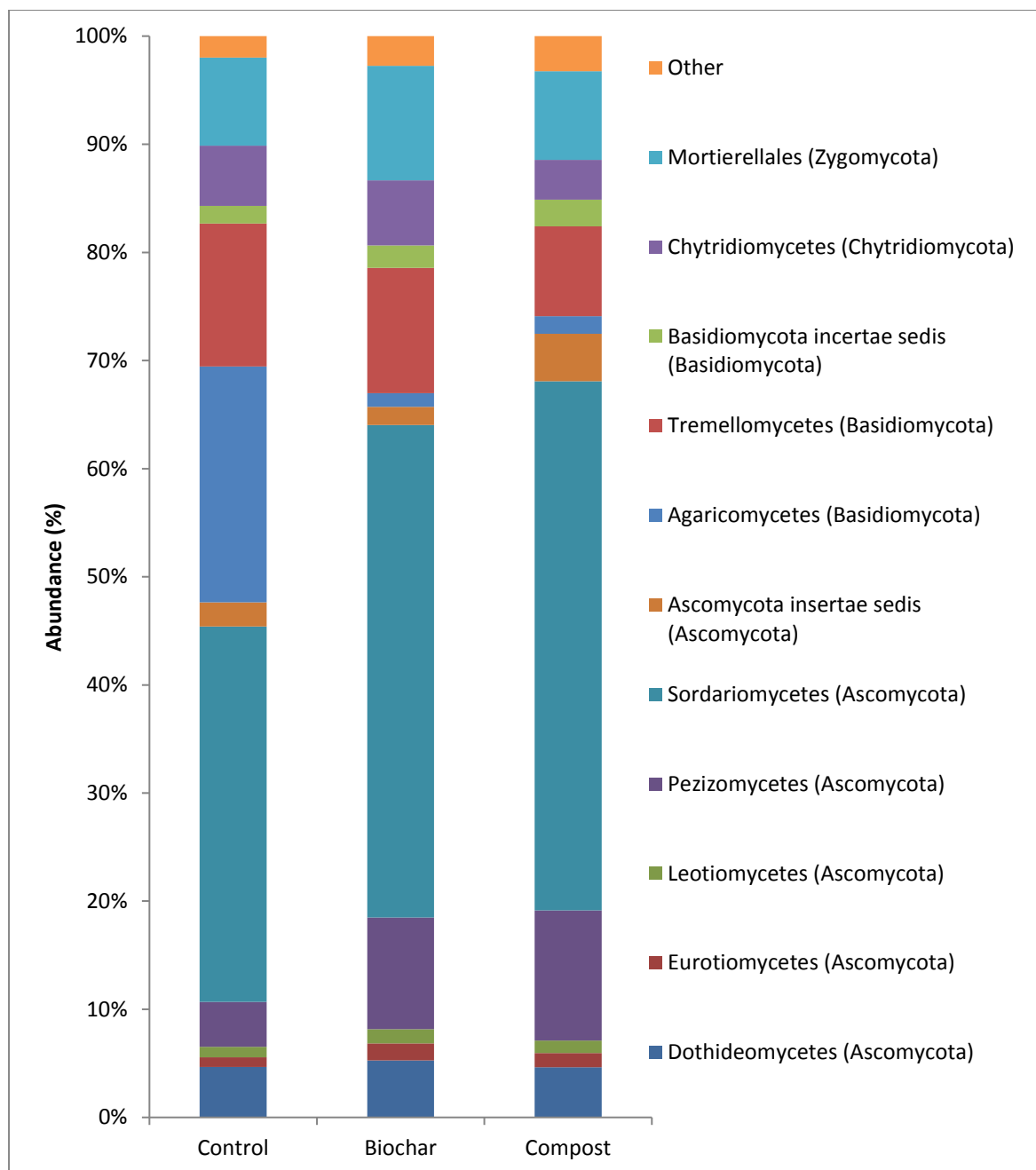


Figure 2.6: Averaged proportion of fungal taxa at the class level in the control, biochar and compost-amended orchard soils as determined by 18S rRNA amplicon sequence analysis.

4. Discussion

4.1. Physical and chemical properties

A prior study of physical properties of the Mountain River orchard site revealed biochar amendment enhanced near-saturated hydraulic conductivity and reduced bulk density (Hardie et al., 2014). This was believed to be due to macropores forming due to greater bioturbation activity (Hardie et al., 2014). However, there was no significant effects on aggregate stability, drainable porosity (between -1.0 and -10 kPa), water content at field capacity or permanent wilting point, and hence plant available water capacity (Hardie et al., 2014). This suggests that direct biochar-influenced soil porosity and water retention changes are very limited but could suggest indirect impacts on these properties due to the alteration of the invertebrate abundance and diversity (McCormack et al., 2013). The different size of biochar pores may make it a habitat occupied by micro and mesofauna (protozoa, nematodes, mites, collembola and enchytraeids), which can contribute to soil structure and aggregation, and this may explain the increase in predominance of nematodes in biochar amended soil. Other organisms such as macrofauna (earthworms and termites) could create larger pores, and thus increase the availability of air-water interfaces in the soil (Lee and Foster, 1991). However, the degree of change in these characteristics is influenced by the type of biochar added to the soil (Chen et al., 2010), and also the type of soil and other organic amendments (Hardie et al., 2014).

The biochar treatment but not the compost treatment was found to increase tree girth in the years one and four following application. However, no effect was found on tree photosynthetic capacity, leaf nutrient levels, daily water use or fruit yield and quality (Eyles et al., 2015). These results correlated to findings here that neither biochar nor

compost had any direct impact on nutrient availability in the orchard soil itself; however both biochar and compost amendments had a strong impact on soil organic carbon levels (Table 1.1). The results support the contention that soil amendments can compensate for loss of organic matter due to agricultural practices and thus could potentially assist in improving physical, chemical and biological properties in soil (Arriagada et al., 2014) indirectly if not directly. The lack of significant changes in any of the key soil fertility indicators such as Colwell phosphorus and potassium, extractable sulphur, soluble nitrate and ammonium and base cations may relate to the regular use of fertigation (weekly) and the annual application of fowl manures to this commercially managed orchard in which the trial was undertaken. However the significant increase in the soil organic carbon in both treatments does indicate that the soil's capacity to retain and release nutrients has been improved.

While other studies have reported an increase in soil pH following biochar application (Kimetu et al., 2008), our results showed a decrease in soil pH after the addition of biochar; and also with compost application. The effect of biochar on soil pH is dependent on the pH of biochar itself and the liming value, which is dependent on the feedstock and pyrolysis conditions used for biochar production (Kookana et al., 2011; Lehmann et al., 2011). As the biochar in this study had a pH of 6.4, it was not surprising that it did not have a liming effect as has been observed in biochars with high pH values. An increase in organic matter following the addition of biochar or compost may also decrease soil pH due to the microbial activity and organic acids released during organic matter decomposition.

4.2. Soil biomass changes

The results of this study indicated that the number of bacteria and the overall biomass was slightly increased in biochar amended soil compared with untreated soil. The increase observed in biochar amended soil agrees with the finding of O'Neill et al. (2009) that microbial abundance is improved after the addition of biochar, however, the effect of compost addition was greater in comparison to the control and biochar plots. Many studies indicate that several factors may be involved in affecting microbial community structure following biochar addition, for example, a shift in pH after biochar application could alter the biodegradation process and microbial community structures (Jones et al., 2011). However, previous studies reported that any pH increase or decrease after biochar application depends on the feedstock and the application rates, which still needs to be investigated. The enhancement of microbial community structure is more likely to be due to the physicochemical characteristics of biochar and compost added to the soil (Saison et al., 2006), although there may also be an indirect impact resulting from changes in nutrient availability that occurred after treatment applications. Addition of biochar to the soil often increases nutrient and water retention and provides suitable habitats for soil microorganisms (Lehmann et al., 2011; Ennis et al., 2012), however, in this system we show that this is not evident although biological modification is detectable. Organic matter and its application play an important role on the soil biodiversity and relevant processes in the soil. Therefore, using biochar as a soil conditioner is considered to be one of the main aspects which affect the soil food-web structure (Moore et al., 2004; Brussaard et al., 2007).

4.3. Enzyme activity

Biochar and compost applications had a limited effect on soil enzyme activity in comparison to the control plot. Generally, the changes in enzyme activities might be a response to carbon and chemical alterations after biochar and compost applications (Kotroczo et al., 2014). Biomass changes were not sufficient to result in substantial change to the overall rates. Nevertheless, many factors may be involved as a result of the wide range of changes in soil chemical and physical properties after biochar amendment. Bailey et al. (2011) reported that biochar pores and nutrient availability may improve root growth and P uptake, and this may explain the slight enhancement of the production of the P mineralising enzyme alkaline phosphatase. Biochar could have a significant impact on microorganisms related to the nutrient transformations in soil, and there could be organisms that are also sensitive to the changes in chemical properties occurring after biochar addition (Lehmann et al., 2011). Although, there were no significant effects on most enzymes activities, biochar may react differently depending on initial soil chemical properties such as pH and CEC (Joseph et al., 2010). The data reflects fundamentally that microbial enzyme function seems to stay relatively stable despite biochar and compost amendments with any potential initial impact having dissipated within the 3.5 year period of the trial.

4.4. Biological community structure alteration

The complexity of soil systems in terms of structure and function is related to the variety of interactions of many taxa including bacteria, archaea, fungi and soil fauna (Atkinson et al., 2010). These groups have a significant impact on soil health and

quality, which also might be affected by addition of organic matter to the soil. Significant differences in bacterial and fungal community structures were observed in the orchard soil studied here after the additions of biochar and compost. The alteration in the fungal community structure was the most highly significant, although a weaker but significant difference was also observed for the overall bacterial community structure. The results also suggested that the proportions of soil eukaryote change to an extent in biochar and compost amended soil. The microbial diversity is rather variable and dependent on the soil properties, for example, microbial communities in Amazonian terra preta are relatively disparate despite being heavily influenced by organic carbon. This is possibly due to temporal and spatial dimensions of the actual carbon amendment process with the result the microbial community has adapted accordingly (O'Neill et al., 2009). To some extent the patchiness of soil microbiota distributions also affect the means by which the sequence data is interpretable (Frey, 2014). This required the application of multivariate analysis and only broad groups (class, phyla) can be realistically compared.

The results of this study showed that the application of biochar and compost affected the microbial communities, but bacterial groups seem to have the same general trends between carbon amendments relative to untreated soil. The changes in the physiochemical properties after biochar and compost application such as pH, water content, CEC and especially the organic carbon might be the main reason for changes to the bacterial populations (Steinbeiss et al., 2009; Lehmann et al., 2011; Kelly et al., 2014). The decline in *Flavobacteriia* suggests a change in the accessibility of utilisable carbon or other nutrient resource in the carbon amended soils. Typically members of this class are copiotrophic chemoheterotrophs which are well studied in aquatic ecosystems (Buchan et al., 2014). Increased competition for carbon and other nutrients

due to the larger soil biomass in the amended soils could result in this group being displaced by various *Proteobacteria*, *Spartobacteria*, and *Rubrobacteridae*, which could be more adept at competing for the altered resource regime. The actual nutrient alterations rendered are unknown and community analysis insufficiently detailed to infer what these changes could be. This understanding is also fundamentally hampered by a general lack of knowledge of soil microbial functionality (He et al., 2012). More research is needed to detail community structural changes in relation to functional changes in biochar treated soils to better ascertain the connection between community changes, the functional outcomes and the actual consequences to soil biology. This requires larger number of replicates, deeper sequencing and importantly, more controlled conditions to account for spatial variability and agricultural management practices.

Moreover, the addition of biochar appeared to change the relative abundance of various soil fauna, especially soil nematodes compared to control and compost treatments. These are even more subject to spatial heterogeneity and the temporal impact produced by the carbon amendments, thus the significance of changes observed tend to be more questionable. Much evidence however suggests that biochar can provide a suitable habitat for certain soil fauna by altering soil porosity and increasing CEC, which increases nutrient base cation availability (Lehmann and Joseph, 2009; Kookana et al., 2011). The actual impact of biochar on soil fauna has to date been investigated rather rarely and has received less attention than microorganisms (Lehmann et al., 2011). Changes to soil fauna observed here, especially soil nematodes and arthropods, might be associated with increased overall abundance of biota in the soil, assumed to be largely bacteria and fungi which are typically prey (Hallmann et al., 1999; Akhtar and Malik, 2000). Though the proportion of annelid sequences seems to be slightly reduced

in the carbon amended soils, it is possible their response could be time dependent. The suggestion that increased bioturbation occurs in the amended soils (Hardie et al., 2014) could be a consequence of activities that dissipate over time or could be seasonal in nature. Thus there is a need to undertake shorter and longer term studies as well as studies in which amendements are applied at different rates and at multiple times.

In this study any apparent increase in density of soil nematodes and other bacterivorous and fungivorous fauna would tend to counteract any increases in bacterial and fungal biomass and activity. This interaction might directly result from predation or indirectly from changes in the nutrient recycling and competition, dependent on the actual nematode density within the environment (Traunsperger et al., 1997), which was not assessed here. The statistical analysis illustrated that the greatest impact occurring from biochar and compost application was on soil fungal community composition. The response of soil fungi to biochar application is different depending on the functions and characteristics of different fungal groups (Atkinson et al., 2010). Generally the biochar properties improve soil fungi colonization and hyphal growth due to higher porosity, nutrient retention and water holding capacity (Lehmann and Joseph, 2009; Lehmann et al., 2011). Previous studies stated that mycorrhizal fungi seemed to be increased after the addition of biochar (Warnock et al., 2007), observed here for compost but not biochar, however the mechanism of this enhancement remains unclear. On the other hand, the biochar and organic application may also cause a decrease in mycorrhizal fungi due to the increase of nutrient availability, especially phosphorus, and modifying soil pH (Gaur and Adholeya, 2000; Warnock et al., 2010). Many studies which support our findings claimed that the compost amendment significantly affected the fungal community structure in soil (Saison et al., 2006; Farrell et al., 2010) likely due to

introduction of labile carbon that is accessible to fungal metabolism, such as complex polysaccharides and lignocellulosic material present in the humus.

5. Conclusion

The results of this study support our hypothesis or aim suggesting that the application of biochar and compost seems to subtly influence soil characteristics leading to changes in bacterial and fungal community structure more than three years after the original application of the amendments. The changes in eukaryote community structure could be associated with enhancement in macroporosity and bioturbation in the soils although it is unknown to what extent the observations change over time and whether the effect of the amendments is stable or in a process of dissipation. The relatively high fertiliser input to the field site potentially masks changes to soil chemical properties. Nevertheless, the application of biochars and composts and their impact on some soil physical and chemical, but particularly biological properties, is visible despite this. It is important to consider that many factors are involved in the impact of biochar and compost application on soil fertility, including the source of organic materials, soil type, fertiliser rate and biochar application rates. A better understanding of the consequences of the additions by connecting practices with outcomes, and understanding the underlying mechanisms driving soil changes over time, will help achieve the maximum benefits and efficient use of biochar and compost in soil.

Chapter 3

The effect of biochar loading rates on soil fertility, soil biomass, potential nitrification and soil community metabolic profiles in three different soils

Abstract

Biochar is increasingly being used as a soil amendment to both increase soil carbon storage and improve soil chemical and biological properties. To better understand the shorter term (10 months) impacts of biochar, a wide range of loading rates were applied to investigate its impact on selected soil parameters and biological processes in three different textured soils. Biochar derived from eucalypt green waste was mixed at 0%, 2.5%, 5%, 10% (wt/wt) with a reactive black clay loam (BCL), a non-reactive red loam (RL) and a brown sandy loam (BSL) and placed in pots exposed to the natural elements. After 10 months incubation, analyses were undertaken including estimates of microbial biomass by total viable counts (TVC) and DNA extraction. Moreover, potential nitrification rates and community metabolic profiles were assayed to evaluate microbial function and biological processes in biochar amended soils. The results showed that biochar additions had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content in both a soil type and loading dependent manner. In the heavier and reactive BCL, no significant impact was observed on available P and K levels, nor total exchangeable base cations (TEB) and CEC. However, in the other lighter soils biochar addition had a significant effect on exchangeable Al, Ca, Mg and Na levels and CEC. There was a relatively limited effect on microbial biomass in amended soils; however, biochar addition and its interactions with different soils reduced the potential

nitrification at the higher biochar rate in the two lighter soils. Community metabolic profile results showed that the effect of biochar on carbon substrate utilisation was both soil type and loading dependent. The BCL and BSL showed reduced rates of substrate utilization as biochar loading levels increased while the opposite occurred for the RL.

This research shows that biochar can improve soil carbon levels and raise pH but its effect is soil type dependent. High biochar loading rates may also influence nitrification and the function and activity of microbial community in lighter soils.

1. Introduction

Biochar has emerged as a commercially available amendment for possible improvement of soil health, physical properties and chemical fertility. It consists of an aromatic stable porous carbon structure which is highly resistant to chemical and microbial degradation compared to other organic materials in soils (Glaser et al., 2001). As such, biochar has been considered as a mechanism for carbon sequestration applicable in long term agriculture practices (Rondon et al., 2005). Biochar amendment in soil may affect the microbial population as a result of changes to microbial activity, biomass and community structure (Ducey et al., 2013). However, the interaction between different biochar application rates and soil characteristics still needs to be further explored (Lehmann et al., 2011). Biochar has a high carbon to nitrogen ratio (C:N ratio) and high surface area that can increase cation exchangeable capacity (CEC) which in turn may enhance nutrient retention and water holding capacity, especially in sandy textured soils (Lehmann et al., 2006).

Biochar characteristics potentially drive changes which occur in soil physical properties including structure, field-texture, porosity, particle size and density. These in turn may affect soil aeration, water holding capacity and microbial activity (Atkinson et al., 2010). Likewise, biochar can influence chemical properties of soil through increased surface area and added labile nutrients. Biochar addition to soil has been shown to alter pH, electrical conductivity (EC), cation exchange capacity (CEC), nutrient retention and nutrient availability (Gundale and DeLuca, 2007). Therefore, with all these possible changes that can accrue in soil because of biochar addition, understanding the interaction between biochar loading rates, soil properties and the microbial community

therein is needed to better model the effect of biochar and its implications on soil function.

The high porosity, surface area and CEC of biochar could improve the habitat for soil microorganisms and plant roots (Atkinson et al., 2010). Application of biochar could also improve moisture retention in lighter soils and water infiltration in heavier soils. Improving hydraulic conductivity and other physical characteristics in soil provides suitable conditions for chemical interactions and subsequent enhancement of microbial activity (Chen et al., 2017). Furthermore, because biochar has high resistance to microbial degradation; the impacts of biochar addition could persist in soil for a long time. The use of biochar should be more beneficial in soils that have poor physical characteristics such as sandy soils. The available evidence in the literature suggests that in some situations biochar can contribute to soil structural stability and aggregation, improve water retention, and increase porosity and surface area. Many studies also indicate that better understanding of biochar effects in different soil types would assist in using it for more effective management of different soil properties and so gain the maximum benefit from its application (Sohi et al., 2010).

The impact of biochar on soil is complicated because of the wide range of biochar effects on soil chemical properties due to variation in biochar chemical and physical features. The effect of soil amendment will depend on the type of biochar and the impact it can achieve in a given timescale (Unger et al., 2011). Biochar amendment and its high surface area is often correlated with CEC enhancement which may increase the availability and use efficiency of plant nutrients in some soils depending on biochar specification. Also biochar can potentially increase pH in soil, which subsequently influences many of the nutrient transformations and their availability to plants (Fowles, 2007). Soil pH may increase or decrease depending on the inherent pH and lime content

of the biochar itself (Lehmann et al., 2011). Biochar and other organic amendments added to soil will probably increase nutrient storage in the rhizosphere in a form that is available to plant uptake (Steiner et al., 2007). Biochar could significantly enhance crop yield and quality by providing nutrient supply and an improved environment to plant growth (Steiner et al., 2007; Unger et al., 2011). Although there is much evidence highlighting advantages in using biochar as a soil amendment, the interaction between biochar and soil needs to be explored to understand the complexity of biochar reactions, especially in different soils utilised in agricultural regions.

Biochar could alter the biological processes in soil such as N mineralisation and nitrification by affecting bacterial communities involved in these processes as well as providing a suitable environment for overall increased microbial activity (Berglund et al., 2004). It has been documented that biochar constitutes a large percentage of the organic carbon in various soils but the exact nature of this component is still not well understood (Zimmerman, 2010). Kolb et al. (2009) studied the effect of biochar addition on microbial biomass and activity by adding biochar to four different soils (Mollisol, Alfisol, Entisol, and Spodosol) at five application rates from 0 to 0.1 kg/kg biochar-soil. The result showed a significant increase in both microbial biomass and activity with increasing application rates. The study also showed similar patterns of biochar impact on microbial biomass, microbial activity and nutrient availability in all four soils but the microbial response was diverse, dependent on the differences of nutrient availability in each soil (Kolb et al., 2009).

Biochar addition has a significant impact on chemical and physical properties which also affect the biochemical processes and microbial functions in soil. However, the relationships between microbial activity, biological processes and changes in chemical and physical properties resulting from biochar addition are still not well understood.

Because biochar may react differently in soils having different physiochemical properties varying the loading rate ought to show how various soil physiochemical and biological properties are subsequently impacted. Thus the aim of this study was to evaluate the effect of different loading rates of biochar in three different soils.

2. Materials and methods

2.1. Soil collection and processing

Three different topsoils (0 – 20 cm depth) were collected for this experiment. Red Loam Dermosol topsoil (RL: clay 21.61%, silt 22.81% and sand 55.58%) was collected from a farm near Cambridge, Tasmania (42° 48.11.77'S 147° 26.22.03'E). Brown Sandy Loam Kurosol topsoil (BSL: clay 10.43%, silt 9.43% and sand 80.14%) was collected from the headland in an apple orchard at Mountain River, located in the Huon Valley region of southern eastern Tasmania (42° 57.2.91'S 147° 55.2.13'E). Black Clay Loam Vertosol (BCL: clay 28.67%, silt 24.35% and sand 46.98%) topsoil was collected from the forested slopes of Mt Nelson near Bend 3 of Mt Nelson Road, located near the University of Tasmania in Hobart, Tasmania (42° 54. 25.92' S 147° 19.22.35 'E). All soils were placed in plastic containers and taken to the laboratory for immediate water content measurements. Subsamples of the soils were kept frozen at -20°C for biological analysis. The remaining soils were prepared by removing gravel before being mixed with biochar for the subsequent pot experiment.

2.2. Biochar specifications

Biochar used in this experiment was sourced from eucalypt green waste (Black Earth Products, Qld, Australia). The biochar was produced in an updraft rotary hearth gasifier operating with a peak temperature of 650 - 750 °C (feedstock dependant) with oxygen limited atmosphere and residence times not longer than 3 minutes (most typically around 100 seconds). A typical chemical profile of this biochar is shown in Table 3.1. Biochar analysis was conducted by Diagnostic and Analytical Services (DAS) in the Department of Primary Industries, Wollongbar NSW 2477 Australia.

Table 3.1: Chemical analysis showing the specification of biochar produced at Black Earth Products, QLD Australia

Parameters	Value
EC (dS/m)	0.27
pH (CaCl ₂)	7.3
Total Nitrogen (%)	0.26
Total Carbon (%)	79
KCl Extractable Ammonium-N (mg/kg)	<0.3
KCl Extractable Nitrate-N (mg/kg)	0.41
CaCO ₃ (%)	2.4
Total Phosphorus (mg/kg)	390
Water Soluble Phosphorus (%)	64
Citrate Insoluble Phosphorus (mg/kg)	230
Citrate Soluble Phosphorus (mg/kg)	90
Available Phosphorus (mg/kg)	150
Aluminium (meq/100g)	< 0.1
Calcium (meq/100g)	9.2
Potassium (meq/100g)	2.9
Magnesium (meq/100g)	1.8
Sodium (meq/100g)	1.1
CEC (meq/100g)	15
Calcium/Magnesium Ratio	5.2
Boron (mg/kg)	6.1
Chromium (mg/kg)	450
Copper (mg/kg)	12
Iron (%)	0.71
Manganese (mg/kg)	180
Sulfur (mg/kg)	80
Zinc (mg/kg)	200

2.3. Experimental design and biochar addition

Biochar was added to the three soils described above at 0%, 2.5%, 5%, 10% (wt/wt), specifically 0, 32.5, 65 and 130 g biochar to a total of 1300 g soil, thoroughly mixed and placed into 1.5 litres pots. Each biochar- soil combination was replicated four times to give a total of 48 pots. Pots were arranged randomly and left exposed to the natural elements for 10 months outside the teaching glasshouse complex at the School of Land and Food at UTAS. The annual mean maximum and minimum temperature is 19.9°C and 8.3°C, respectively, while the mean annual rainfall is 613.3 mm (Hobart Ellerslie Road Weather Station, Lower Derwent Tasmania).

2.4. Chemical and physical analyses

After 10 months incubation, soil samples were taken from the pots for gravimetric water content measurements and chemical analysis. Soil chemical analysis was conducted by CSBP Laboratories, Western Australia. Properties analysed included organic carbon (Walkley-Black), total nitrogen, available phosphorus (Colwell), available potassium (Colwell), sulphur (KCl 40), ammonium nitrogen, nitrate nitrogen, electrical conductivity, pH (H₂O) 1:5, pH (CaCl₂) 1:5, micronutrients (DTPA: copper, zinc, manganese, iron) and boron (Hot CaCl₂), exchangeable cations (calcium, magnesium, sodium, potassium, aluminium) and particle size (pipette and sieving method). Chemical and physical data were statistically analysed by Multivariate analysis of variance (MANOVA) using SPSS v22 to assess the effect of biochar addition on soil properties compared to the unamended control treatments.

2.5. Soil biomass assessment

Soil bacterial numbers as total viable counts (TVC) were estimated from colony forming units (CFUs) on 10% tryptone soy agar plates incubated at 25°C for 21 days (Juhnke et al., 1987). Soil biomass was estimated from extracted DNA (n=4 replicates for each treatment) with quantities estimated via the Nanodrop spectrophotometer instrument (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE 19810 U.S.A.) which is used as an alternative method to estimate microbial biomass (Marstorp et al., 2000; Bouzaiane et al., 2007).

2.6. Potential nitrification assay

Potential nitrification was assayed by using ammonium sulphate as a substrate as described by Kandeler (1995), soil samples from each treatment replicate were incubated at 25°C for 5 h. After incubation, the nitrite released during the incubation was extracted with potassium chloride and determined using spectrophotometry at 520 nm using a SPECTRO star Nano plate reader (BMG Labtech, Mornington, VIC Australia).

2.7. Soil community level metabolic profiles

Microbial metabolic capacity of the soil communities was determined by examining the ability to utilize a variety of carbon sources. This was done using Biolog EcoPlates as described by Garland and Mills (1991) and Frac et al. (2012). Eco Plates with 96 wells containing 31 triplicated carbon sources and a control (water) were prepared by

suspending 1 g soil in 99 ml sterilised water, and 120 µl of each soil suspension then aliquoted into each well. Plates were incubated at 25°C for 7 days. Absorbance at 590 nm from all 96 wells was measured daily using a SPECTROstar Nano plate reader. The data was compiled and analysed in the program PRIMER 6 + PERMANOVA (Primer-E Ltd, Plymouth UK) using analysis of similarity (ANOSIM), canonical analysis of principal coordinates (CAP) and permutation analysis of variance (PERMANOVA). For this the data was square root transformed and converted to Bray-Curtis similarity values. ANOSIM was used for *a priori* analysis of factors (biochar loading, soil type, time of incubation) followed by PERMANOVA analysis using unrestricted permutation of the data with 9999 permutations assuming a type III (unbalanced) design. The rate and pattern of the overall microbial carbon source utilisation was expressed by the average well-colour development (AWCD), richness (R) and Shannon–Weaver index (H) (Garland, 1997; Gomez et al., 2004). To reduce the complexity of interpreting the data, carbon substrates were divided into five groups: (1) carbohydrates; (2) carboxylic and acetic acids; (3) amino acids; (4) polymers; and (5) amines and amides according to Weber and Legge (2009) and presented as a percentage of the total absorbance values for each treatment.

3. Results

3.1. Chemical and physical properties

The addition of different rates of biochar to different soils had a significant impact on selected chemical and physical properties in soil. These differences were dependent on the soil type and the amount of biochar added to that soil.

3.1.1. Ammonium and nitrate nitrogen in soil

The interaction between soil type and biochar loading rate had a significant negative impact on exchangeable ammonium ($p=0.02$) and positive impact on soluble nitrate ($p=0.012$) contents. In the BCL, exchangeable ammonium decreased by 32%, 53% and 61% respectively with increasing biochar loading rates (2.5%, 5% and 10%) compared to the untreated control as shown in Fig. 3.1A. The $\text{NO}_3\text{-N}$ content increased dramatically by 103%, 110% and 207% with increasing biochar loading rates (Fig. 3.1B). To a lesser extent the same trends were observed with the BSL and RL soils, especially when the ammonium levels were higher in the 10% biochar applications. However, no significant differences in soluble nitrate contents were observed between the biochar treatments in the BSL or RL topsoils.

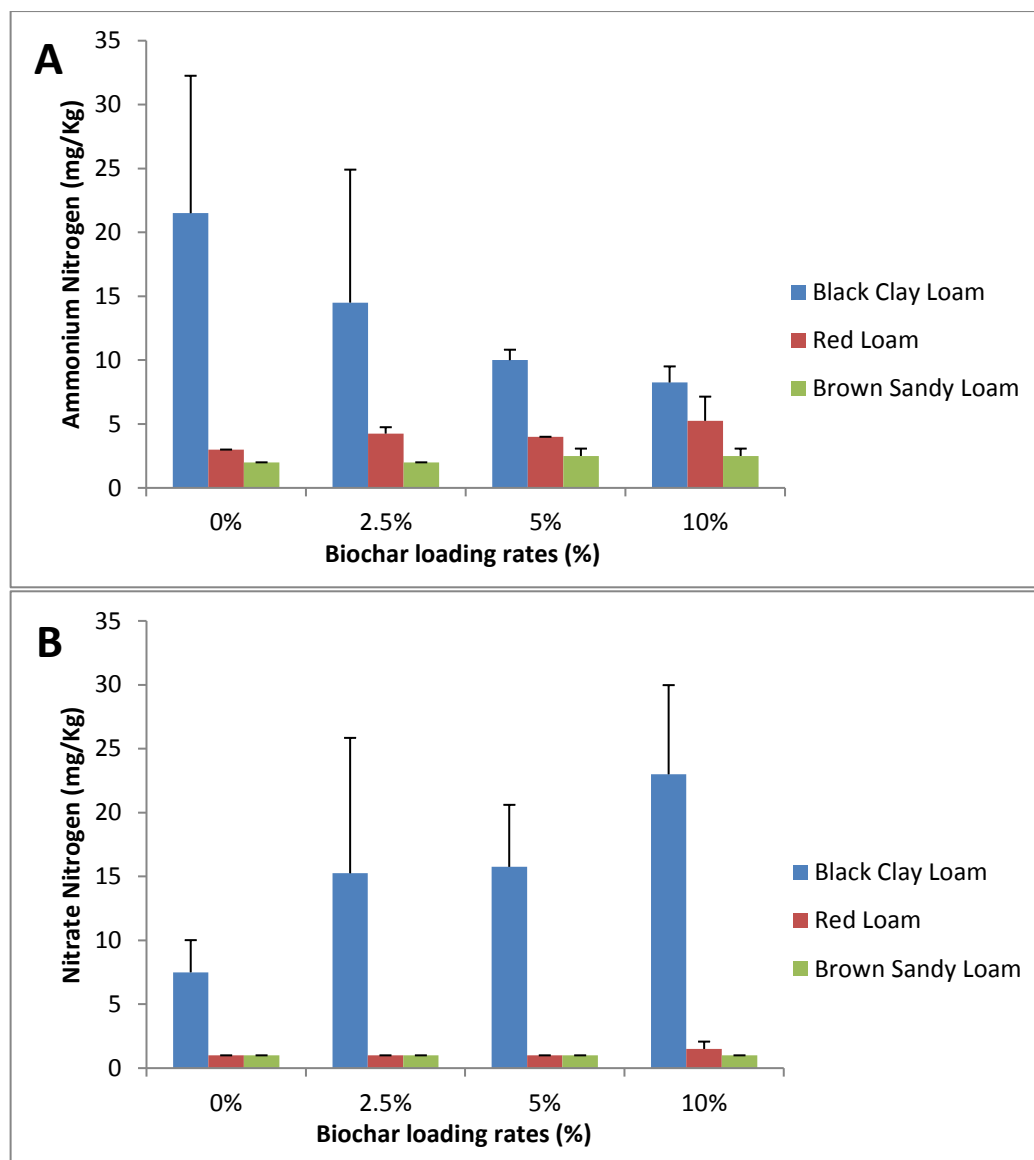


Figure 3.1: (A) ammonium nitrogen (mg/kg) and (B) nitrate nitrogen (mg/kg) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Errors bars are standard deviation from four replicate soil pots.

3.1.2. Total carbon and total nitrogen

Biochar loading rates had a very significant effect on the total carbon in all three soils ($p < 0.001$). Total carbon increased with increasing amount of biochar added to each soil as shown in Fig. 3.2A. There was no significant effect on total nitrogen in the BCL or the BSL after the biochar applications, whereas the results illustrated that the additions of different loading rates of biochar to the RL significantly reduced total nitrogen ($p = 0.007$) content after 10 months (Fig. 3.2B).

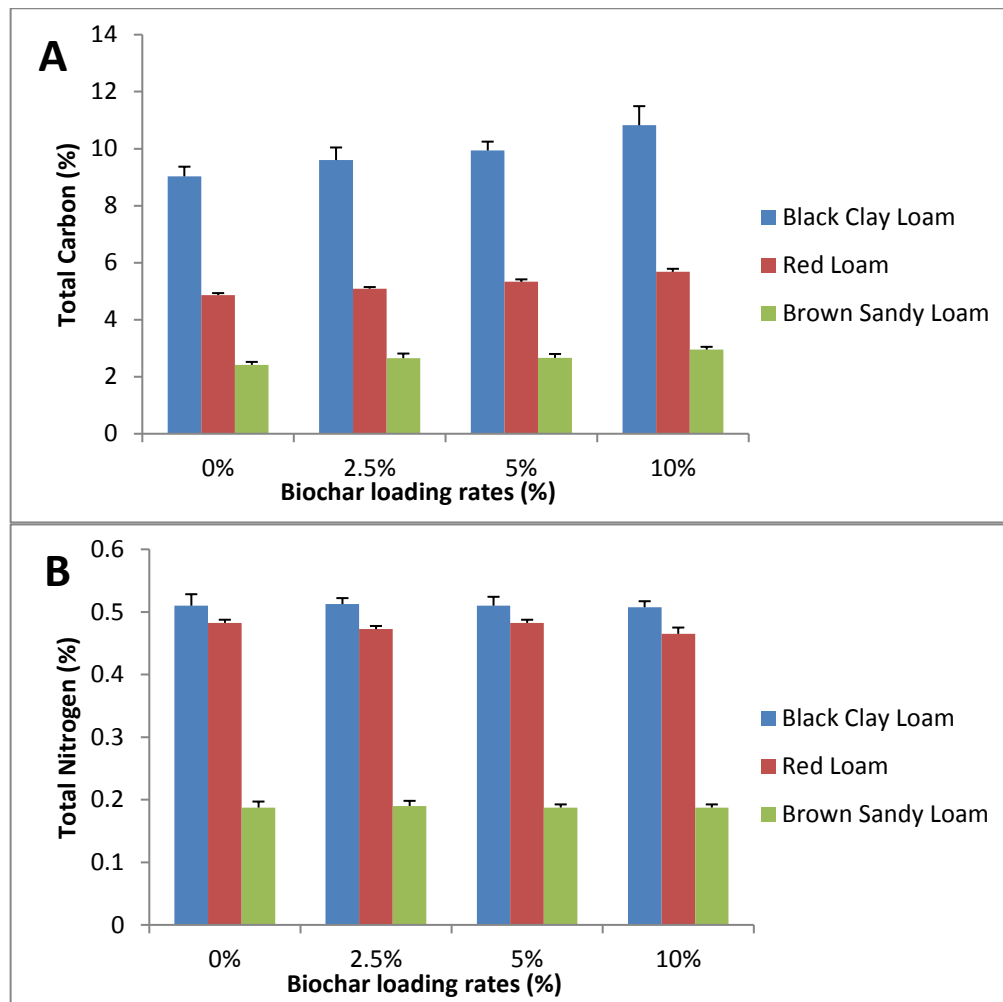


Figure 3.2: (A) Total carbon (%) and (B) total nitrogen (%) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Errors bars are standard deviation from four replicate soil pots.

3.1.3. Colwell phosphorus and potassium

There was no significant impact of biochar loading rate on Colwell phosphorus or potassium in the RL, however biochar addition did affect phosphorus in the BSL ($p=0.041$) and potassium in the BCL ($p=0.028$) (Fig. 3.3). In the BSL, Colwell phosphorus decreased with the 2.5% and 5% biochar treatments but increased with the 10% biochar treatment compared to the untreated control (Fig. 3.3A). Colwell potassium content in the BCL increased gradually from 174 mg/kg in the control treatment (biochar 0%) to reach 204 mg/kg at the highest level of biochar loading (10%).

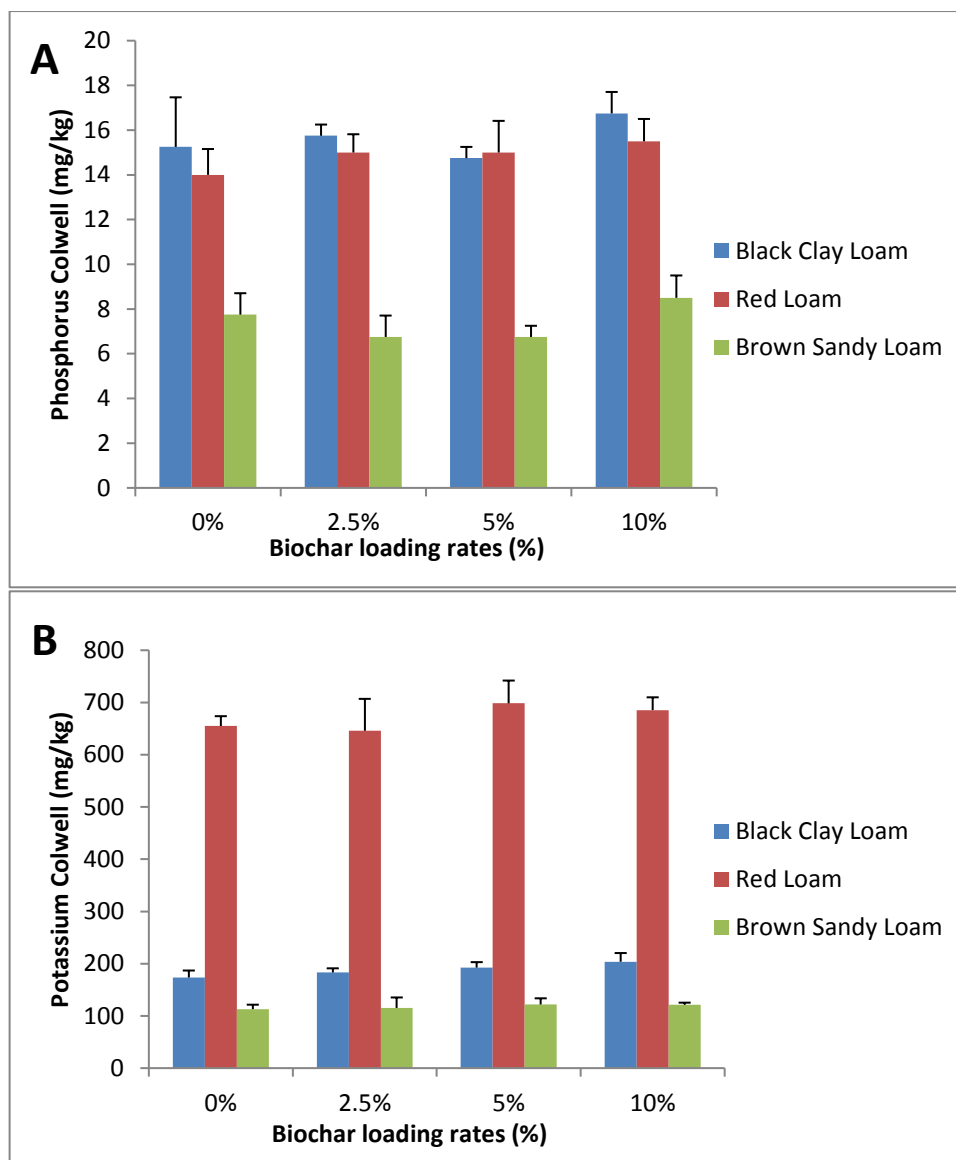


Figure 3.3: (A) Colwell phosphorus and (B) Colwell potassium (mg/kg) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Errors bars are standard deviation from four replicate soil pots.

3.1.4. Soil pH and electrical conductivity

Soil pH (Fig. 3.4) was significantly increased in the BSL, increasing from pH (CaCl₂) 4.50 in the control treatment to 4.60, 4.72 and 4.83 at the 2.5%, 5% and 10% biochar loading rates respectively ($p=0.001$). The same pattern was found in the RL where pH level was 4.60, 4.67, 4.90 and 4.98 at the 0%, 2.5%, 5% and 10% biochar rates, respectively ($p<0.001$). In the BCL the effect of biochar loading rate was less significant ($p=0.037$) compared to the BSL and RL soils. The pH level increased from 4.90 at the 0% biochar treatment to 5.00 at the higher loading rates 10% biochar. No significant differences were observed in EC in the BCL or the BSL while a slight decrease was observed in the RL within the biochar treatments ($p=0.049$) as shown in Fig. 3.5.

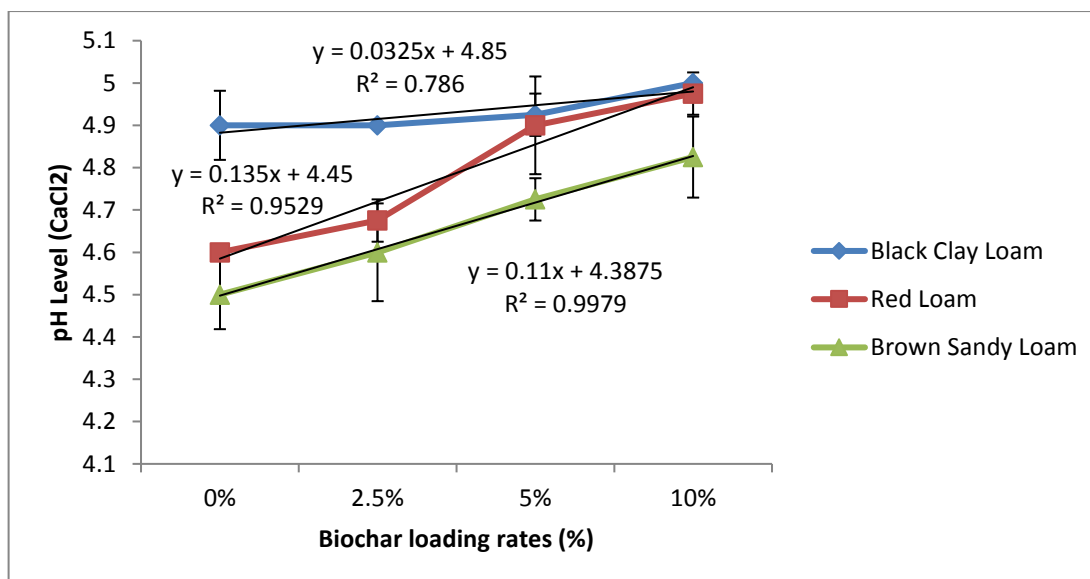


Figure 3.4: Soil pH (CaCl₂) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Errors bars are standard deviation from four replicate soil pots.

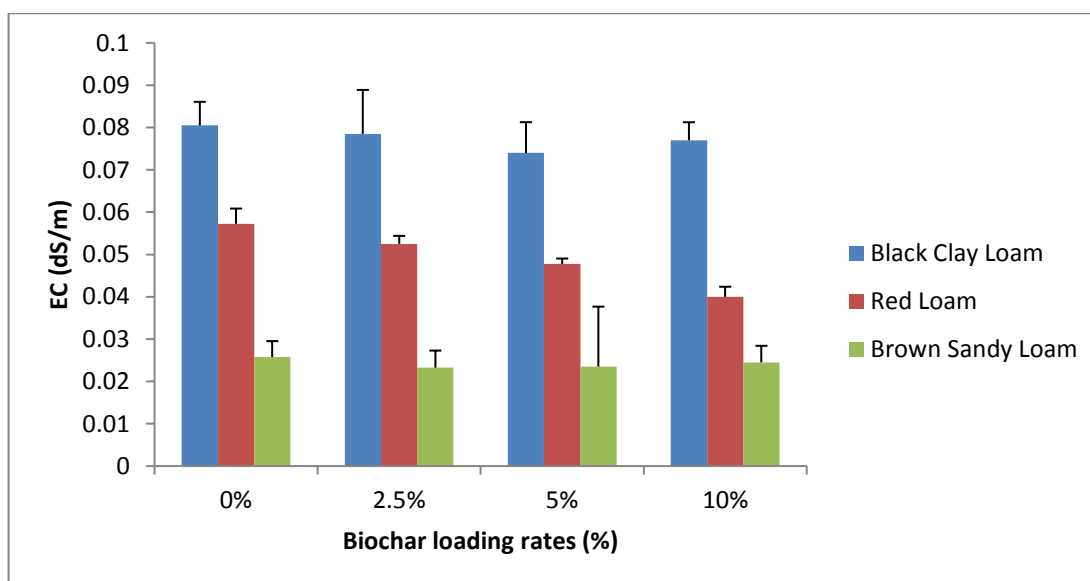


Figure 3.5: Electric conductivity (dS/m) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Errors bars are standard deviation from four replicate soil pots.

3.1.5. Soil moisture content

There was a significant impact of different biochar levels on water content in all three soils ($p=0.016$), especially at 10% biochar application in the RL and BSL (Fig. 3.6). Soil water content was increased by 10% in the BCL at the higher rate of biochar, while no significant increase was observed at 2.5% and 5% biochar. The BSL and RL showed the same trend where the water content was increased by 22% in the BSL and by 19% in the RL at 10% biochar compared to the corresponding control.

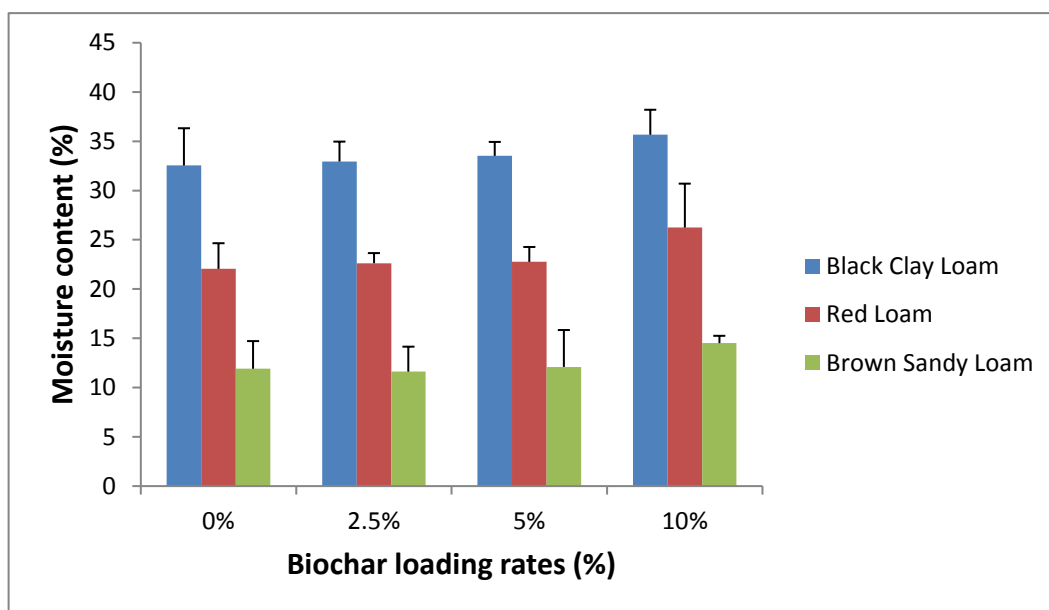


Figure 3.6: The effect of biochar loading rates (0 to 10% wt/wt) on moisture content (%) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil. Errors bars are standard deviation from four replicate soil pots.

3.1.6. Exchangeable cations and cation exchangeable capacity (CEC)

The results in Table 3.2 show no significant effects were measured in the level of exchangeable cations in the BCL except for potassium which increased significantly by 8% and 16% at 5% and 10% biochar application levels ($p=0.001$). On the other hand, exchangeable aluminium, calcium and sodium were significantly affected by the addition of different biochar levels in both the BSL and the RL. Exchangeable calcium and sodium were increased by 19% and 28% in the BSL and by 9% and 14% in the RL respectively at 10% biochar application levels, whereas exchangeable aluminium decreased by 68% in the BSL and by 66% in RL at 10% biochar rates compared to the untreated control. Exchangeable magnesium increased ($p=0.02$) in the RL soil with the higher biochar loading rates, but no significant impact was found in either the BCL or the BSL. The results in Table 3.2 also indicated that biochar addition had a potential impact on the CEC. The main differences were found in the BSL where the CEC increased by 4% at 2.5% biochar treatment, 7% at 5% biochar and 14% at 10% biochar loading rate. CEC was slightly increased in the RL mainly in the 10% biochar treatment while no differences were found between biochar application rates in the BCL.

Table 3.2: The effect of biochar loading rates (0 to 10% wt/wt) on exchangeable cations (Ca, Mg, Na, K, Al), and cation exchange capacity (CEC) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil.

Soil Type	Biochar loading rates (%)	Exc. Al (meq/100g)	Exc. Ca (meq/100g)	Exc. Mg (meq/100g)	Exc. K (meq/100g)	Exc. Na (meq/100g)	CEC (meq/100g)
BCL	0%	0.119	21.01	9.43	0.42	0.29	31.14
	2.5%	0.108	20.71	9.29	0.42	0.26	30.67
	5%	0.111	20.67	9.27	0.45*	0.25	30.65
	10%	0.097	21.11	9.29	0.48**	0.26	31.14
RL	0%	0.158	8.99	2.68	1.51	0.14	13.33
	2.5%	0.085**	9.42	2.74	1.48	0.14	13.77
	5%	0.089**	9.44	2.80**	1.53	0.15	13.93*
	10%	0.054**	9.81**	2.77*	1.51	0.16*	14.24**
BSL	0%	0.186	2.91	1.34	0.21	0.07	4.54
	2.5%	0.134**	3.05	1.37	0.23	0.08	4.73
	5%	0.091**	3.19**	1.37	0.23	0.09**	4.88**
	10%	0.060**	3.48**	1.39	0.23	0.09**	5.19**

** High significant between means (p<0.01), * significant between means (p<0.05)

3.1.7. Micronutrients

The manganese level (Table 3.3) was decreased by biochar application in BCL ($p=0.044$) and in the BSL ($p<0.001$). Biochar addition had no significant impact on manganese in the RL. Zinc increased with increasing biochar level in both the black clay and sandy loams ($p<0.001$) but no differences were observed in the RL. Copper and iron content in soils varied dependent on soil type and biochar level. In the BCL, no significant impact was found for copper while iron was significantly different between biochar treatments as shown in Table 3.3. Iron increased by 6% in the 2.5% biochar treatment and decreased by 13% at 10% biochar rates (Table 3.3). Significant differences were found for copper ($p=0.019$) and iron ($p=0.002$) in the BSL, copper decreased by 16% while iron decreased by 19% at 10% biochar level compared to the control. No significant effect was found on iron content in the RL, however slight differences in copper were found ($p=0.038$) between biochar treatments, with copper reduced by 10% in the RL at 10% biochar treatment compared to the untreated control (Table 3.3). Biochar loading rate had a highly significant impact ($p<0.001$) on boron in the BSL but no differences were found in the other two soils. The results in Table 3.3 show that boron increased by 19% at 2.5 biochar, 32% at 5% biochar and by 40% at 10% biochar application in the BSL.

Table 3.3: The effect of biochar loading rates (0 to 10% wt/wt) on soil micronutrients (Mn, Zn, Cu, Fe, B) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil

Soil Type	Biochar loading rates (%)	DTPA Mn (mg/kg)	DTPA Zn (mg/kg)	DTPA Cu (mg/kg)	DTPA Fe (mg/kg)	Hot CaCl ₂ B (meq/100g)
BCL	0%	58.07	20.93	2.71	191.09	1.05
	2.5%	45.51	21.98	2.66	201.88	0.97
	5%	40.71	23.21	3.01	191.93	1.06
	10%	34.12*	25.81**	3.08	167.18*	1.05
RL	0%	37.06	6.23	3.19	197.78	0.97
	2.5%	35.43	11.84	3.23	204.54	0.96
	5%	34.27	9.45	3.08	212.79	0.95
	10%	32.23	18.04	2.86*	190.81	1.01
BSL	0%	9.43	2.68	2.62	108.39	0.29
	2.5%	7.41*	3.74	2.26	100.47	0.34*
	5%	6.49**	4.26*	2.21*	98.15	0.38*
	10%	5.43**	6.38**	2.20*	87.37*	0.40**

** High significant between means (p<0.01), * significant between means (p<0.05)

3.2. Microbial biomass

The total viable count (TVC) results (Fig. 3.7) showed no significant effect of biochar loading rate for the BCL. Furthermore, no effect was observed on TVCs in BSL at the two lower biochar rates but the addition of biochar at 10% reduced TVCs compared to controls. On the other hand, biochar addition enhanced TVCs at 2.5% and 5% but not the 10% biochar levels in the RL compared to the untreated control. The higher rate of biochar reduced TVC in the BSL soil.

Soil microbial biomass was also estimated from the amount of DNA extracted at the beginning of the experiment and after 10 months incubation with the different levels of biochar (Fig. 3.8). The amount of DNA extracted from the BCL and BSL was higher in the 10% biochar (approximately 21 ng / μ l for both soils) compared to initial samples (17.6 ng / μ l from BCL and 13.0 ng / μ l from BSL) and 0% biochar treatments (19.2 ng / μ l from BCL and 16.32 ng / μ l from BSL). The amount of DNA extracted from the RL soil was significantly higher compared to the BCL and BSL, the higher quantity of DNA was observed in soil taken from the 0% biochar (25.0 ng / μ l) to 5% biochar (26.2 ng / μ l) compared to initial samples.

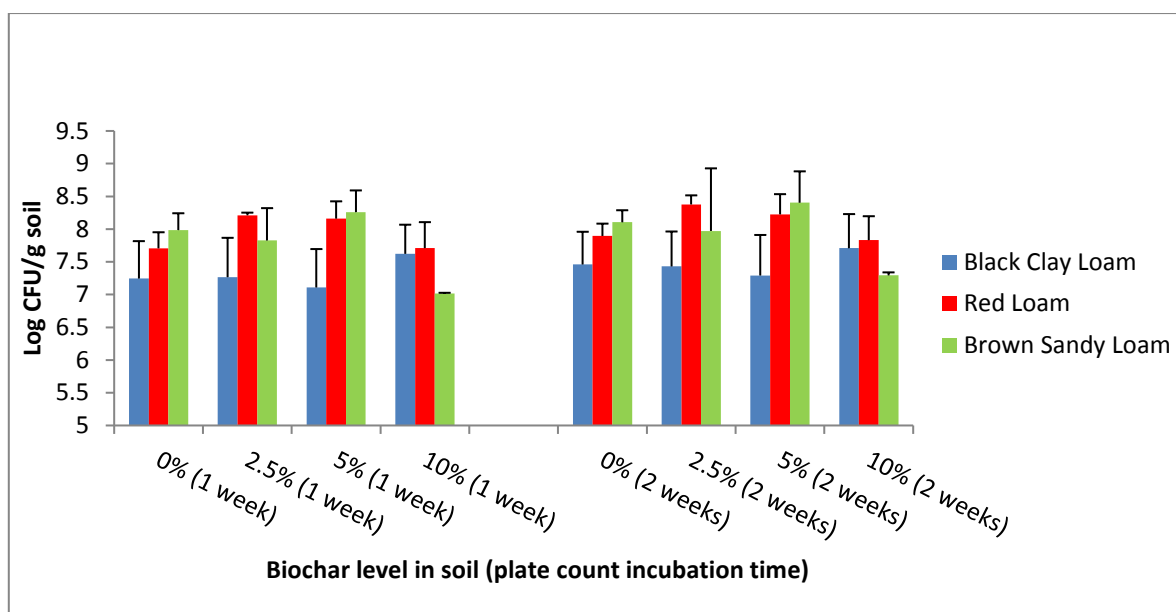


Figure 3.7: Microbial enumeration of bacteria in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Counts are from plates assessed after 1 week and after 2 weeks. Errors bars are from counts from three replicate soil pots.

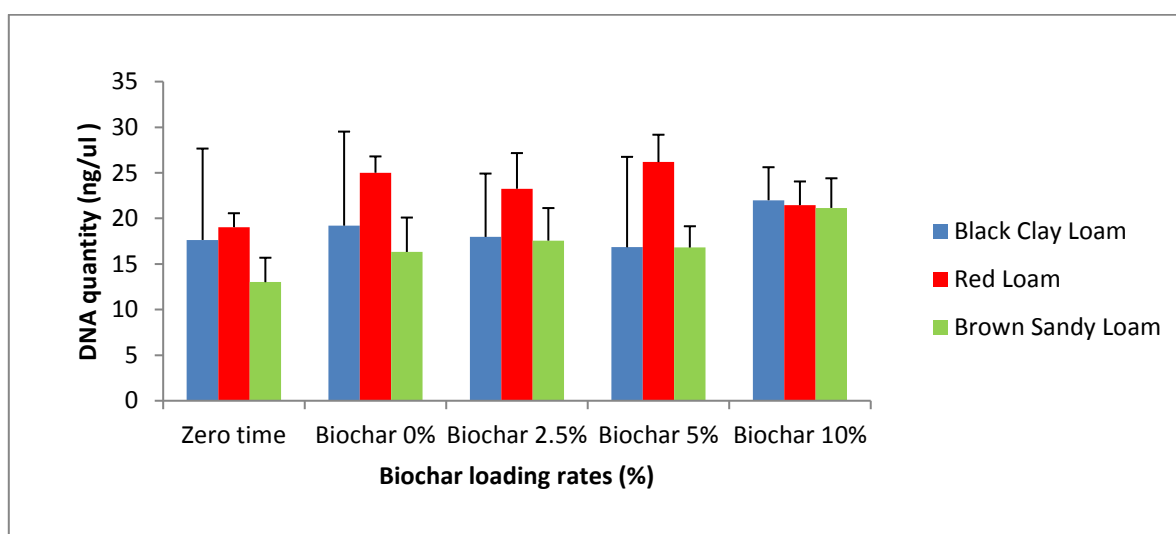


Figure 3.8: Soil microbial biomass as DNA extracted from a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt) with quantities estimated via the Nanodrop spectrophotometer. Errors bars are standard deviation from four replicate soil pots.

3.3. Potential nitrification

Biochar and its interaction with the different soils had a significant impact on potential nitrification, which was used to estimate the abundance and activity of the ammonium oxidizer bacteria and archaea ($p=0.011$). Generally, the BSL showed the highest nitrification rates compared to BCL and RL except at the 10% biochar treatment level (Fig. 3.9). Potential nitrification seemed to have the same patterns in the RL and BSL where nitrification rates increased at the 2.5% biochar treatments compared to control (0% biochar). However, no significant differences were observed between different biochar loading rates in the BCL.

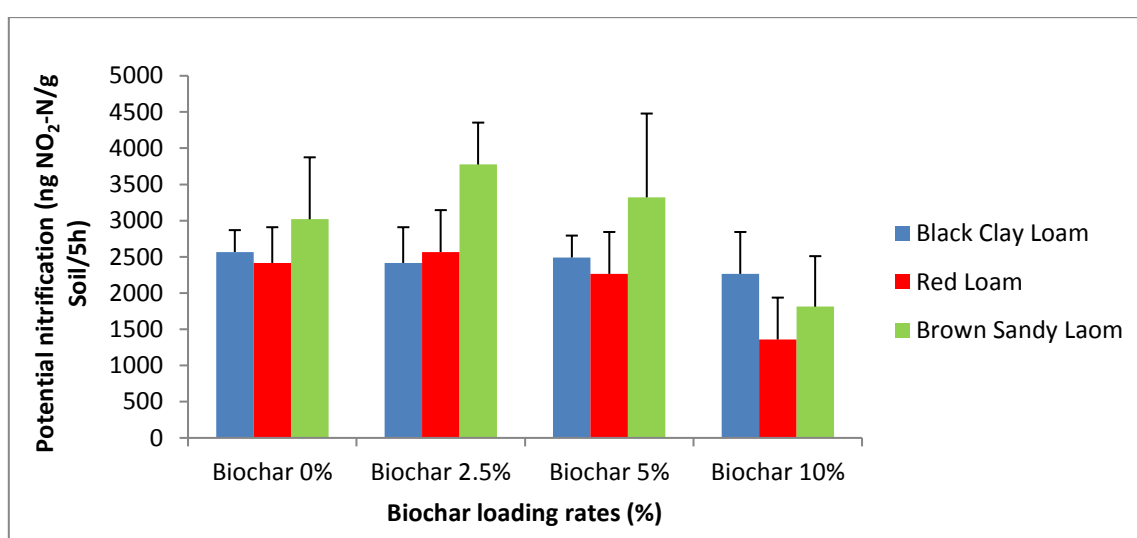


Figure 3.9: Potential nitrification rates (ng NO₂-N/g Soil/5h) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil containing different biochar loading rates (0 to 10% wt/wt). Errors bars are standard deviation from four replicate soil pots.

3.4. Community level metabolic profiles

Biolog Ecoplates were used to evaluate whether biochar loading rate and soil type influence microbial communities in the utilization rates of different carbon substrates. PERMANOVA analysis revealed significant differences between substrate utilisation in relation to biochar loading rates in a soil-type dependent manner. Both biochar loading rate and soil type strongly interacted ($p=0.0001$, $F=5.59$) while no interaction occurred with time of incubation ($p > 0.13$). The BCL and BSL showed reduced rates of substrate utilization as biochar loading levels increased while the opposite occurred for the RL. In the latter case, there was pronounced increase in the utilisation of plant decomposition substrates and lipid analogs, including α -cyclodextrin, cellobiose, xylose, methyl- β -glucoside, Tween 40 and Tween 80. The AWCD (Fig. 3.10B) and R (Fig. 3.10C) showed the same patterns where the utilisation of C substrates declined with increasing biochar loading rates in the BCL and BSL compared to the control, however, C utilisation in the RL increased with increasing rate of biochar compared to the control. No significant differences were observed between treatments in terms of number of substrates utilised estimated from the Shannon–Weaver index (H) in all the trial soils (Fig. 3.10A), however, there was strong correlation between H, AWCD and R in the BCL ($r=0.65-0.92$). There was also strong correlation between AWCD and R in the RL ($r=0.91$) and BSL ($r=0.98$). A comparison between the major five carbon substrate groups as shown in Fig. 3.11 indicated that the carbon utilisation had the same patterns among treatments in all soils, suggesting that there are no large changes occurring in the microbial community. However, there are difference in carbon utilisation especially in the amines and amides in BCL (Fig. 3.11A) and polymers in the RL (Fig. 3.11B) compared to the control which may indicate that biochar addition changes the functional diversity among treatments.

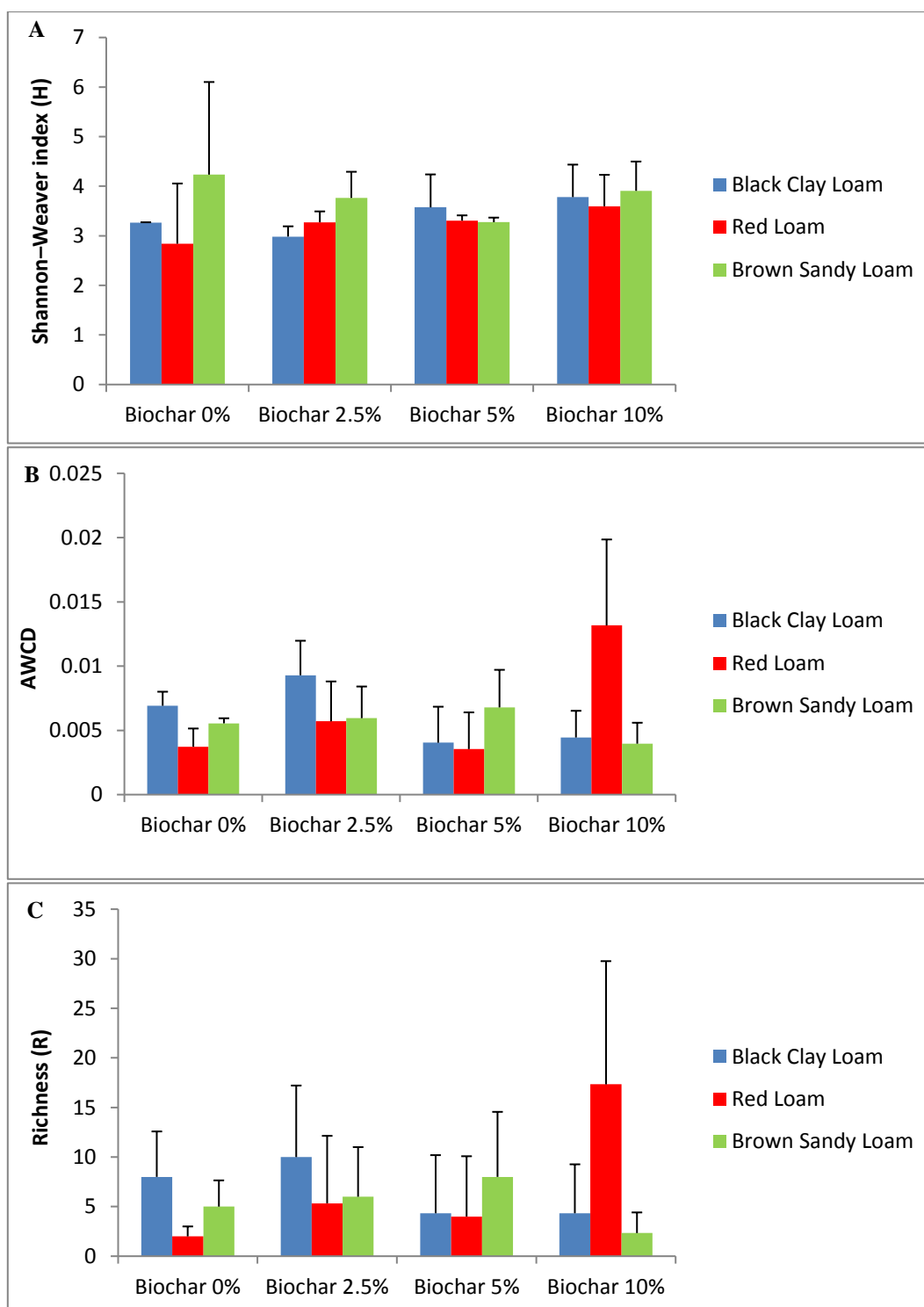


Figure 3.10: The effect of biochar loading rates (0 to 10% wt/wt) on carbon source utilization presented as (A) Shannon–Weaver index (H), (B) Average well-color development (AWCD) and (C) Richness (R) in a Black clay loam (BCL), Red loam (RL) and Brown sandy loam (BSL) soil. Errors bars are standard deviation from three replicates.

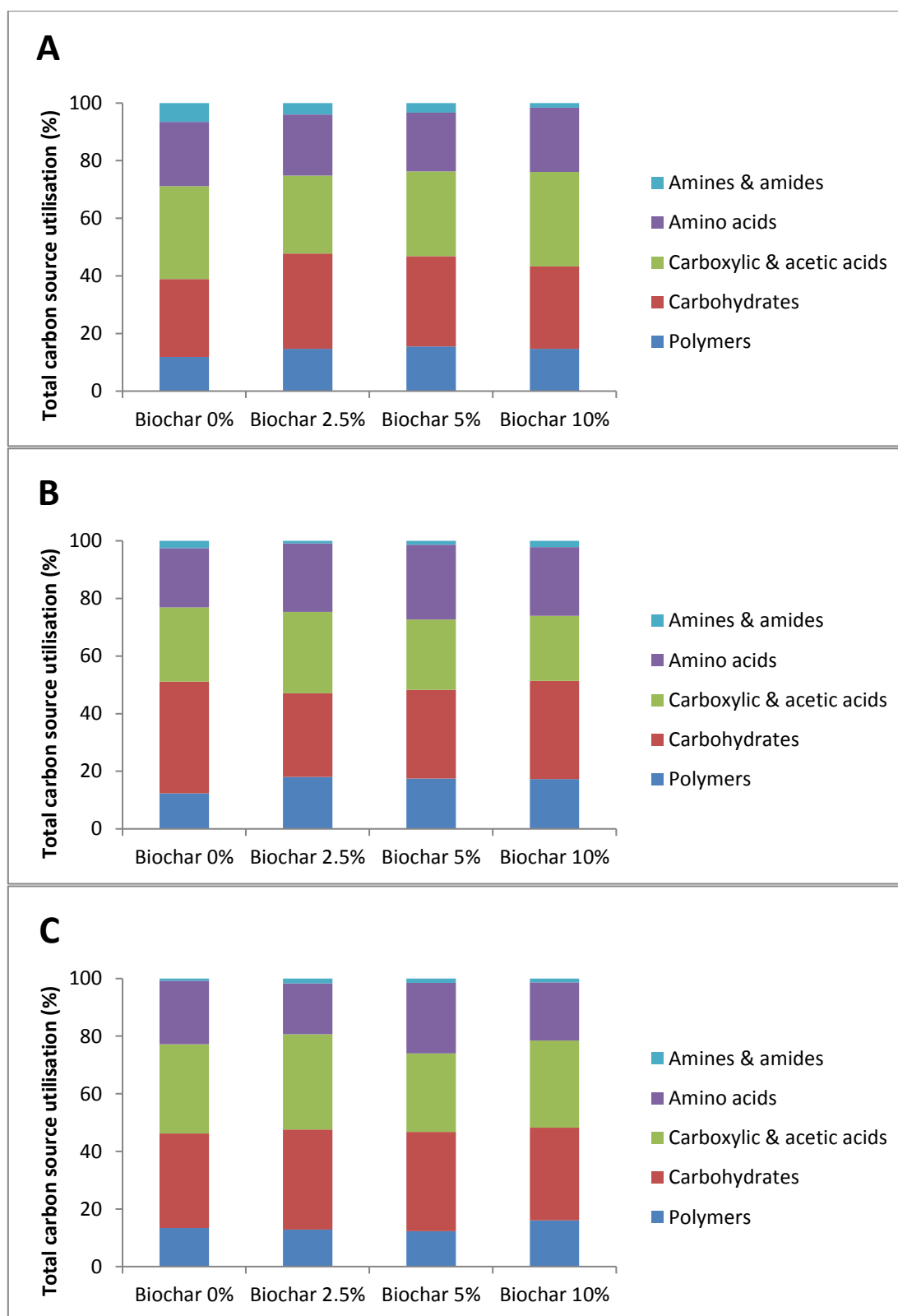


Figure 3.11: Comparison of total carbon source utilisation between amines & amides, amino acids, carboxylic & acetic acids, carbohydrates and polymers in (A) Black clay loam (BCL), (B) Red loam (RL) and (C) Brown sandy loam (BSL) soil containing different levels of biochar (0 to 10% wt/wt).

4. Discussion

Although only one biochar type was examined in this study, the results have shown that biochar loading rate can affect soil physical, chemical and biological properties, but is also influenced by soil type.

4.1. Soil chemical parameters

Biochar application had a significant impact on soluble ammonium and nitrate content in the three soils as measured at the end of the 10 month experiment. The ammonium decreased while nitrate increased with increasing biochar rate. This might be explained by improved soil conditions in the 10% biochar loading, including measured improvements in soil pH and soil moisture content, resulting in little mineralisable ammonium present after 10 months as it was all converted to nitrate. While a potential nitrification test showed that the highest biochar rates had significantly lower nitrification rates in the two lighter soils, nitrification was otherwise unaffected or increased with biochar. Biochar produced by higher temperature ($> 600\text{ }^{\circ}\text{C}$) has the potential to adsorb both NH_4^+ and NO_3^- ions due to net negative and positive surface charges on biochar (Dempster et al., 2012b). However, it is commonly reported that most biochars have a dominantly negative surface charge and hence NH_4^+ retention capacity (Lehmann et al., 2011). The ability of biochar to adsorb elements will more likely depend on the nutrients and biochar properties (Yao et al., 2012).

The increase in total carbon with increasing application rates in all soils was predictable as a result of the high C input with biochar application having a C:N ratio of 303. According to Lehmann et al. (2011) and Nelson et al. (2011), addition of biochar tends to rapidly increase decomposition rates of labile soil carbon, which requires utilisation of soil N; this may be the

reason for the observed reduction in total N in the RL. There was also a significant decrease in the organic carbon, as measured by Walkley-Black method, in the BC at all rates of biochar.

The results also showed an improvement in CEC after biochar addition in the two lighter soils (RL and BSL) which has been reported in the literature (Uzoma et al., 2011). However, there was no consistent improvement in available soil phosphorus and potassium with biochar amendment. The effect of biochar on nutrient availability is influenced by biochar types (feedstock and pyrolysis time and temperature) and its interaction with different soils. An experiment conducted by Uzoma et al. (2011) to investigate the impact of cow manure biochar on maize yield, nutrient uptake and physio-chemical properties of a dryland sandy soil showed that cow manure biochar significantly increased soil pH, total C, total N, Olsen-P, exchangeable cations and CEC in the soil. The lack of any consistent positive impacts of biochar loading rate on available P and K levels observed in our study probably relates to the initial P and K content in this soil and the low P and K levels in the biochar.

It is been widely documented that biochar amendment increases soil pH (Kimetu et al., 2008; Chintala et al., 2014). The results of this study indicated that biochar additions increased soil pH with increased loading rates, especially in the RL and BSL soils. This increase in soil pH with increasing biochar application rates was expected due to the alkalinity of the biochar used in this study (Chintala et al., 2014). Biochar amendment had no impact on EC except for the strongly structured RL where EC was consistently reduced. This supports our suggestion of greater leaching potential in the more strongly structured soils, i.e., ammonium leaching.

The impact of biochar and its interactions depend on biochar types and reactions in different soils (Kookana et al., 2011; Lehmann et al., 2011) and its capacity to adsorb selective nutrients (Novak et al., 2009a). The biochar additions may affect micronutrient content in soil

due to their sensitivity to changes in soil pH, soil porosity and aeration. Soil aggregation may benefit from biochar additions to clay soils while sandy soils are also improved by the surface area increases associated with biochar (Basso et al., 2013; Ulyett et al., 2014).

Our data showed that both available Fe and Mn generally decreased in the soils with increasing biochar applications. These decreasing trends were most significant in the heavier BCL and BSL soils, and this may relate to improved aeration and drainage in the soil as biochar rates increased causing soluble iron and manganese oxides to precipitate. Also Kumar and Babel (2011) indicated that micronutrients correlated positively with organic C in soil but negatively with CaCO_3 and soil pH. The biochar used in this experiment is both alkaline and slightly calcareous.

Both copper and zinc were increased at the higher biochar application rates in the BCL soil. Zinc also increased in both RL and BSL at all rates, but not copper. In fact copper was reduced in those soils with the greatest pH increase. Zinc levels were 200 mg/kg in the biochar and this might well explain the uniform increases measured. We interpret the mixed story of some increases and some decreases in micro-nutrients as relating to the relative impacts of biochar induced changes in pH, carbon and calcium carbonate levels as well as the unmeasured impacts on the soils aeration and porosity. Hence it is not unreasonable to expect that the modifications were induced by biochar amendments as they modify the soil environment and the content and availability of micronutrients in soil.

4.2. Microbial biomass

Previous studies demonstrated that biochar addition to soil enhances microbial abundance and activity (O'Neill et al., 2009; Bamminger et al., 2014; Gomez et al., 2014). However, our results showed limited impact of biochar on microbial biomass, at least in the short term. Noyce et al. (2015) studied soil microbial responses over 1 and 2 years following biochar addition to a temperate forest soil, indicating that biochar had an inconsequential impact on bacterial and fungal community composition, fungi/bacteria ratios, and microbial biomass. The increase of soil pH after biochar addition normally is expected to enhance microbial abundance in soil (Lehmann et al., 2011), however, biochar addition at high application rates seemed to reduce microbial biomass which may be related to the reductions in organic matter decomposition and N availability (Dempster et al., 2012a). The finding of this experiment showed a reduction in N content with increasing biochar loading rates in RL which might be as a result of N consumption by microorganisms during organic matter decomposition (Lehmann et al., 2011; Nelson et al., 2011). The initial decomposition might be increased after biochar addition to soil due to additional C and higher pH, then was limited by N deplete by soil microorganism. While there is no fertiliser input, the nutrient content might be reduced in soil and affect microbial abundance and activity. There are many factors involved in the impact of biochar on microbial abundance and activity in addition to biochar C in amended soil (Gomez et al., 2014). Carbon input following biochar application proportionally affects microbial growth and activity; but the degree of change is likely to be dependent on the initial soil nutrient content and C levels. This would explain the difference on biochar effects observed in the different soils studied.

4.3. Potential nitrification

It has been well documented that biochar amendment to soil has the potential to improve nitrification and consequently increase N availability to plants (Berglund et al., 2004; Ball et al., 2010; Prommer et al., 2014). However, there is a risk of losing NO_3^- through leaching from soil and denitrification activity. Biochar is considered to be beneficial in stimulating nitrification and at the same time reducing N loss due to its physical and chemical specifications (Dempster et al., 2012b; Yao et al., 2012). Our findings support this with potential nitrification increased in biochar amended soils, especially in the BSL soil at the 2.5% and 5% biochar loading rates. The nitrification enhancement and the biochar sorption capacity are likely to have contributed to the reduction in NH_4 and increase in NO_3 with increasing biochar loading rates. Nitrification seemed to be inhibited at the higher level of biochar amendment which may be related to the high C/N ratio (Bengtsson et al., 2003) followed biochar amendment which in turn led to inorganic N immobilisation and reduction of ammonium oxidation activity (Song et al., 2014). The impact of biochar on the C and N pools in soil may vary depending on the biochar properties. As stated by Zhang et al. (2015), biochar produced at high temperature ($> 400^\circ\text{C}$), such as used in this study, has the potential to add more stable C to the soil thus decreasing CO_2 emission, and increasing NH_4 sorption capacity and soil pH and CEC. However, biochar produced at lower temperatures provides labile C useable by microorganism and that may temporarily enhance biological N transformation. Therefore, biochar specifications should be taken into consideration to maximise the benefit of biochar when used as a soil amendment. Biochar produced at high temperature may improve N usage efficiency, nutrient retention and alter N and C transformations (Zhang et al., 2015).

4.4. Community Level Metabolic Profiles

It has been well documented that carbon availability plays an important role in microbial growth and activity (Alden et al., 2001; Yoshitake et al., 2007). Therefore, application of organic amendments not only affects the soil chemical and physical properties, it also enhances microbial function and activity related to nutrient cycling (Ros et al., 2006). Biochar is a very stable form of carbon which is resistant to microbial degradation, thus the available carbon used by soil microorganisms is more likely to be from different sources. A study conducted by Dempster et al. (2012a) showed that the addition of 25 t ha⁻¹ biochar altered the community metabolic profiles, however, the change in the ammonia oxidising bacterial community occurred only when a source of N was combined with biochar application. The results here showed a decrease in microbial activity through the reduction of organic matter decomposition and N mineralisation in soil, which might be due to the decrease in microbial biomass. This may explain the decline in the C source utilisation in the BCL and BSL soils. However, the metabolic profile in the RL increased with increasing biochar rates, possibly due to the presence of other complex carbon sources present in this soil. The native grass and root residues in the RL soil might explain the enhancement in C substrate utilisation. Baudoin et al. (2003) stated that the root exudates stimulate bacterial growth and increase microbial community. Different plant species and residues as organic compounds in soil may increase the potential metabolic diversity (Baudoin et al., 2003).

5. Conclusions

The results of this study indicate that the interaction between different soil types and biochar loading rates had a significant impact on the NH₄-N and NO₃-N content, especially in BCL

soil. A highly significant effect was observed on total C in all three soils with no impact observed on total N except in the RL soil. Soil pH was significantly increased with increasing biochar loading rates. No significant impact was observed on P and K availability, total exchangeable base cations (TEB) and CEC in BCL. However, biochar addition had a significant effect on exchangeable Al, Ca, Mg and Na in both the BSL and the RL soils. Biochar addition had a limited effect on microbial biomass, however, the interaction between biochar and different soils significantly affected the potential nitrification especially in RL and BSL soils. There were significant differences in C substrates utilisation among biochar treatments in the three different soils. The BCL and BSL showed reduction in substrate utilization rates as biochar levels increased while the opposite occurred for the RL soil, which indicated that biochar may influence the function and activity of the microbial community. These results show that biochar can improve soil carbon content and increase pH but these vary in different soils. High biochar loading rates may also influence nitrification and the function and activity of microbial community in lighter soils. More studies are required for better understanding of the interaction between biochar application and soil fertility under different conditions and variables including biochar types and application rates, different soils, fertiliser inputs and long-term application.

Chapter 4

Assessment of bacterial community composition, methanotrophic and nitrogen cycling bacteria in three soils with different biochar application rates

Abstract

The increased use of biochar as a soil amendment to alleviate the impact of agricultural practices on climate change has been a motivation for many studies to determine the effects of biochar on soil properties, particularly the abundance and activities of soil microbes and related biological processes. This study investigates the impact of different application rates of wood-derived biochar on community structure, nitrogen cycling and methanotrophic bacteria in three soil types.

Biochar was added at 0%, 2.5%, 5% and 10% wt/wt to black clay loam (BCL, Vertosol), red loam (RL, Dermosol) and brown sandy loam (BSL, Kurosol) soils. Soil chemical analysis and 16S rRNA gene amplicon sequencing using the Illumina Mi-Seq platform were conducted on initial samples and after 10 months incubation.

The results indicated that the addition of biochar loading levels to the different soils had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content. These changes were reflected in significant differences in the bacterial diversity between biochar treatments in the BSL and RL soils, while the BCL soil was more resilient to change. Complete ammonia oxidizing (Nitrospira) and nitrite oxidizing bacteria (NOB) were more abundant than standard ammonia oxidizing bacteria (AOB) in all soils. Increased biochar loading raised the abundance of nitrifying bacteria in BCL soil while Nitrospira became more

abundant in BSL soil. Biochar addition affected the abundance of certain N₂-fixer groups in a soil dependent manner. Strong positive correlations were observed in *Rhizobium* ($r=0.99$) and *Azospirillum* abundance ($r=0.70$) with increased biochar loading rates in BCL. Greater biochar loading also significantly increased the relative abundance of methanotrophs, especially in BCL soil.

The impact of biochar on community structure and nitrogen cycling bacteria depended on soil types and biochar rates which correlated to the differences in soil properties. Overall, the abundance of nitrogen cycling bacterial groups seemed to be most affected by the changes in soil conditions, including aeration, C/N ratio, nutrients and pH in relation to biochar application in different soils. These changes show short term biochar loading influences community structure and leads to increases in populations of methanotrophic and nitrifying bacteria.

1. Introduction

The increased use of biochar as a soil amendment to alleviate the impact of agricultural practices on climate change has been a motivation for many studies to determine the positive and negative impacts of biochar on soil properties, particularly the abundance and activities of soil microbes and related biological processes (Lehmann et al., 2011). The main benefit of biochar application is the ability to increase carbon storage and enhance soil fertility (Glaser et al., 2002). However, understanding the interaction between biochar and biological processes including the direct impact on soil microbes is still limited. Biochar may significantly change the surface area, porosity and sorption capacity of soil (Kookana et al., 2011), which alters appropriate habitats for soil microorganisms.

Previous studies indicated that biochar amendment enhances microbial populations and activity in soil (Kookana et al., 2011; Abujabhah et al., 2016a), although these effects are likely to be associated with changes in soil properties, specially soil pH, C/N ratio and CEC after biochar application. The addition of different biochars also needs to be considered (Chan et al., 2008). Specific biochars can influence physical and chemical characteristics in soil (Gundale and DeLuca, 2007; Atkinson et al., 2010), which may affect the abundance and activity of soil microbes. The complexity of all these factors involved requires a better understanding in order to estimate the behaviour and reaction of soil microbes to better inform biochar related applications. The impact of biochar addition on soil quality varies depending on soil types and application rates. Kolb et al. (2009) studied the effect of biochar addition on microbial biomass and activity in four different soils (Mollisol, Alfisol, Entisol, and Spodosol) at five different rates from 0 to 0.1 kg/kg⁻¹ biochar-soil. Their results indicated that biochar increased the biomass and activity with increasing biochar loading. However, the impact was still variable among the different soils tests, which was correlated with the nutrient availability in each soil.

Different biochars, application rates and soil types may have various influences on microbial communities. Biochar also may enhance the nitrogen input in soil by affecting biological nitrogen fixation. Beck (1991a) demonstrated that biochar potentially affects *Rhizobium* survival in soil and enhanced the growth and nodulation of plants by *Rhizobium* in biochar amended soil.

The forms and availability of the nitrogen play an important role in soil fertility and agricultural production. Nitrogen transformation processes in soil (immobilisation, mineralisation, nitrification and denitrification) ordinarily are affected by microbes, and changes in soil microbial community structure and activity can dramatically influence the nitrogen status in soil. Ball et al. (2010) conducted an experiment to examine the influence of

fire history on the total and potential nitrification rates and the nature and abundance of ammonia oxidizing bacteria in forest soil. This study showed that a 12 year old wildfire resulted in higher content in soil charcoal and also nitrification rates compared with soils impacted by older wildfires in other sites. Greater abundances of ammonia oxidizing bacteria were also observed compared with control soils, which might be the main reason for increased nitrification rates. Biochar can alter the biological processes related to the nutrient cycles by affecting the microorganisms involved in these processes as well as providing suitable environments that result in increased microbial activity (Berglund et al., 2004).

The aim of this study was to determine the impact of biochar loading rates on bacterial communities in different soils and to better understand the interactions between different factors involved in the biology and fertility of biochar-amended soils. To do this a pot experiment was conducted to investigate the impacts of biochar on selected soil parameters and biological processes in three different textured soils. Previous results from this experiment (Abujabhah et al., 2016b) indicated that biochar can improve soil carbon content and increase pH but results differed with soil type. High biochar loading rates also influenced nitrification and the function and activity of microbial communities, especially in the lighter black sandy loam soil (Abujabhah et al., 2016b). This work investigates the impact of biochar application rates on bacterial community composition, methanotrophic and nitrogen cycling bacteria in the three different amended soils.

2. Materials and methods

2.1. Soil collection and biochar specifications

Soil samples were collected from a previous experiment conducted by Abujabhah et al. (2016b) to investigate the impact of different loading rates of biochar in three different acidic

topsoils: black clay loam Vertosol (BCL), loamy red Dermosol (RL) and brown sandy loam Kurosol (BSL). Biochar used in this experiment was sourced from eucalypt green waste (Black Earth Products, Qld). The biochar was produced in an updraft rotary hearth gasifier operating with a peak temperature of 650 - 750 °C (feedstock dependant) with oxygen limited atmosphere and residence times not longer than 3 minutes (most typically around 100 seconds).

2.2. Experimental design and biochar addition

Biochar was added at 0%, 2.5%, 5%, 10% (wt/wt) to the three different soils (BCL, RL and BSL), specifically 0, 32.5, 65 and 130 g to total of 1300 g soil. The biochar and soil combination was mixed and filled into 1.5 litres pots exposed to the natural elements for 10 months. The annual mean maximum and minimum temperatures were 19.9°C and 8.3°C respectively, while the mean annual rainfall is 613.3 mm according to Hobart (Ellerslie Road) Weather Station, Lower Derwent, Tasmania. Each biochar- soil treatment was replicated four times and randomly arrayed in a total of 48 pots.

2.3. Soil and biochar chemical analysis

Soil samples were taken from the pots after 10 months incubation for instant water content measurements and chemical analysis. Soil chemical analysis was conducted by CSBP laboratories, Western Australia. Selected soil parameters were analysed including C (Walkley-Black extract), total N (Elemental Analyser - Leco), available P and K (Colwell extract), S (KCl at 40°C extract), NH₄-N, NO₃-N, EC, pH (1:5 Soil:H₂O extract), pH (0.01 M

CaCl₂), micronutrients (DTPA extract) and B (Hot CaCl₂ extract), exchangeable cations and particle size (pipette and sieving method) to estimate the potential changes in the three soils after biochar amendments. These results were reported previously in Abujabhah et al. (2016b). Biochar analysis was conducted by Diagnostic and Analytical Services (DAS) in the Department of Primary Industries, Wollongbar NSW 2477 Australia.

2.4. DNA extraction, 16S rRNA gene amplicon Illumina MiSeq sequencing and bioinformatics analysis

DNA was extracted from soil at the initial time before incubation and after 10 months using PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc) by following the provided protocol. Extracted DNA (n=4 replicates for each treatment) was checked for quality at 260/280 nm and quantified via Nanodrop spectrophotometer (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE 19810 U.S.A.). Sequence analysis was performed at the Ramaciotti Centre for Genomics (Kensington, NSW, Australia) where the 16S rRNA genes were amplified using 12 bp tagged universal primers 27F (AGAGTTTGATCMTGGCTCAG) -519R (GWATTACCGCGGCKGCTG) (Lane et al., 1985; Lane, 1991; Caporaso et al., 2012). Sequencing was performed using the Illumina MiSeq platform according to standard protocols generating 300 bp pair-ended reads. All reads were filtered based on quality scoring, trimmed of the tag regions, chimera checked using QIIME. 16S rRNA gene sequence reads were classified and binned using the BaseSpace cloud server (<https://basespace.illumina.com/>) 16S rRNA Metagenomics App (Illumina Corp. Proprietary 15055860A). In this process reads were classified systematically to phylum, class, order, family, genera and species against the curated Greengenes 16S rRNA reference (McDonald et al., 2011) database – May 2013 update using an adaptation of the

Bayesian algorithm devised by Wang et al. (2007). The sequence data obtained in this study were deposited in the EMBL database under the accession number: PRJEB8837.

2.5. Statistical analysis

Soil chemical parameters were statistically tested using SPSS v22 to assess the effect of biochar additions on soil properties comparing to an unamended control treatment. PRIMER6 and PERMANOVA+ (version 6.1.12 and version 1.0.2; Primer-E, Ivybridge, UK), respectively were used to conduct permutation multivariate analysis of variance (PERMANOVA) (Anderson et al., 2005), and canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003). Distance-based linear models (DistLM) and distance-based redundancy analysis (dbRDA) were also used to assess the microbial community compositions and the interaction with soil parameters in biochar amended soils. For sequence read analysis, the data was organised at the lowest taxonomic level possible (usually genus to family) and normalised as percentages, square root transformed and a resemblance matrix created by calculation of Bray-Curtis coefficients. PERMANOVA was conducted using default settings with 9999 permutations, while CAP was conducted using default settings. The PERMANOVA derived significance values were considered significant when $P < 0.01$, while $0.01 < P < 0.05$ were considered only marginally significant.

3. Results

3.1. Soil and biochar physicochemical properties

Soil sample analysis previously investigated in experiments detailed by Abujabhah et al. (2016b) focussed on changes in soil chemistry and broad biological factors (soil biomass and

substrate utilisation). The essential findings indicated that the addition of different rates of biochar to different soils had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content. No significant impacts were observed on soluble P and K or exchangeable cations or CEC in BCL soil except exchangeable K; however; biochar addition had a significant effect on exchangeable Al, Ca, Mg and Na in BSL and RL soils (Abujabhah et al., 2016b). Basic parameters of the soil and biochar used in this study are illustrated in Table (4.1).

Table 4.1: Selected parameters in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) soils amended with different rates of biochar (0%, 2.5%, 5% and 10%) and the wood-derived biochar tested in this study.

	Total N (%)	Total C (%)	NH₄-N (mg/Kg)	NO₃-N (mg/Kg)	pH (CaCl ₂)	EC (ds/m)	CEC (meq/100g)
BCL-0%	0.51	9.03	21.5	7.5	4.9	0.080	31.14
BCL-2.5%	0.51	9.60*	14.5	15.2**	4.9	0.078	30.67
BCL-5%	0.51	9.94**	10**	15.8**	4.9	0.074	30.65
BCL-10%	0.50	10.82**	8.25**	23**	5.0*	0.077	31.14
RL-0%	0.48	4.87	3	1	4.6	0.057	13.33
RL-2.5%	0.47	5.09*	4.25	1	4.7*	0.052	13.77
RL-5%	0.48	5.33*	4	1	4.9**	0.048*	13.93*
RL-10%	0.47	5.68**	5.25	1.5*	4.9**	0.040*	14.24**
BSL-0%	0.18	2.42	2	1	4.5	0.026	4.54
BSL-2.5%	0.19	2.65*	2	1	4.6*	0.023	4.73
BSL-5%	0.18	2.66*	2.5	1	4.7**	0.024	4.88**
BSL-10%	0.18	2.95**	2.5	1	4.8**	0.025	5.19**
Biochar	0.26	79	0.3	0.41	7.3	0.27	15

** Highly significant between means (p<0.01), * significant between means (p<0.05)

3.2. Microbial diversity

A total of 7,320,603 sequence reads for 16S rRNA Illumina pyrosequencing were obtained from the soil with an average of 122,010 per sample. The bacterial diversity (Shannon index) was higher in the initial sample compared to the control and biochar treatments in all three soils (Table 4.2). Significant differences were observed in the bacterial diversity between biochar treatments in the BSL and RL soils and slightly in BCL. However, Shannon index values show that the diversity increased in the BSL soil with increasing biochar loading rates compared to the control (0% biochar). The number of OTUs was higher in the RL soil with an average of 1075 per sample compared to the BCL and BSL soils with an average of 951 and 929 respectively. Similar to Shannon index data, the numbers of OTUs were higher in the initial samples compared to the biochar treatments as shown in Table 4.2. No differences were observed in the number of OTUs between the biochar treatments and the control except in BSL where the OTU numbers increased in the highest level (10%) of biochar addition compared to the control. PERMANOVA and CAP analysis as shown in Fig. 4.1 indicated that biochar application rates significantly affect the bacterial community structure in RL ($F=4.5601$, $p=0.0005$) and BSL ($F=2.4464$, $p=0.0005$), however, less impact was observed in BCL ($F=1.8054$, $p=0.0136$) compared to the other amended soils. Selected soil parameters measured in the amended soils explained the total variation in the microbial community by 44.2% in BCL, 51% in RL and 35.1% in BSL soil (Fig.4.4). The changes in the microbial community were strongly correlated to the selected soil chemical properties ($R^2=0.76$ in BCL, $R^2=0.87$ in RL and $R^2=0.68$ in BSL) in all amended soils. However, the soil variables seemed to be more associated with the microbial community changes induced by biochar treatments compared to the control in the lighter BSL soil, especially with pH (CaCl_2), total C, C:N ratio and CEC. While in the heavier soils the microbial community strongly correlated to NH_4 , NO_3 and pH (CaCl_2) in the BCL and NH_4 , total C, pH (CaCl_2) in the RL amended soil.

Overall, the results of DistLM and dbRDA analysis indicated that changes in microbial community structure were strongly associated with the selected soil chemical parameters in the amended soils.

Table 4.2: Bacterial diversity indices (average \pm SD): Shannon index, Operational Taxonomic Units (OTUs) and the number of sequence reads in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) at different loading rates of biochar.

Soil	Treatments	Shannon Species Diversity	OTUs	Number of reads
BCL	Initial sample	2.51 (± 0.09)	1010 (± 17)	122176 (± 18396)
	Biochar 0%	2.49 (± 0.05)	934 (± 20)	120964 (± 11690)
	Biochar 2.5%	2.47 (± 0.02)	924 (± 27)	115849 (± 9514)
	Biochar 5%	2.44 (± 0.04)	946 (± 29)	134840 (± 26244)
	Biochar 10%	2.40 (± 0.04)	943 (± 13)	127991 (± 7564)
RL	Initial sample	2.64 (± 0.06)	1133 (± 40)	125078 (± 13755)
	Biochar 0%	2.41 (± 0.03)	1048 (± 14)	126008 (± 8759)
	Biochar 2.5%	2.44 (± 0.03)	1073 (± 41)	133759 (± 10894)
	Biochar 5%	2.44 (± 0.06)	1061 (± 47)	129274 (± 11344)
	Biochar 10%	2.42 (± 0.03)	1062 (± 17)	124339 (± 10520)
BSL	Initial sample	2.46 (± 0.04)	972 (± 35)	114210 (± 13489)
	Biochar 0%	2.25 (± 0.03)	915 (± 21)	120166 (± 8245)
	Biochar 2.5%	2.27 (± 0.02)	889 (± 24)	114221 (± 12268)
	Biochar 5%	2.31 (± 0.06)	894 (± 27)	107040 (± 10021)
	Biochar 10%	2.41 (± 0.04)	977 (± 6)	114236 (± 4679)

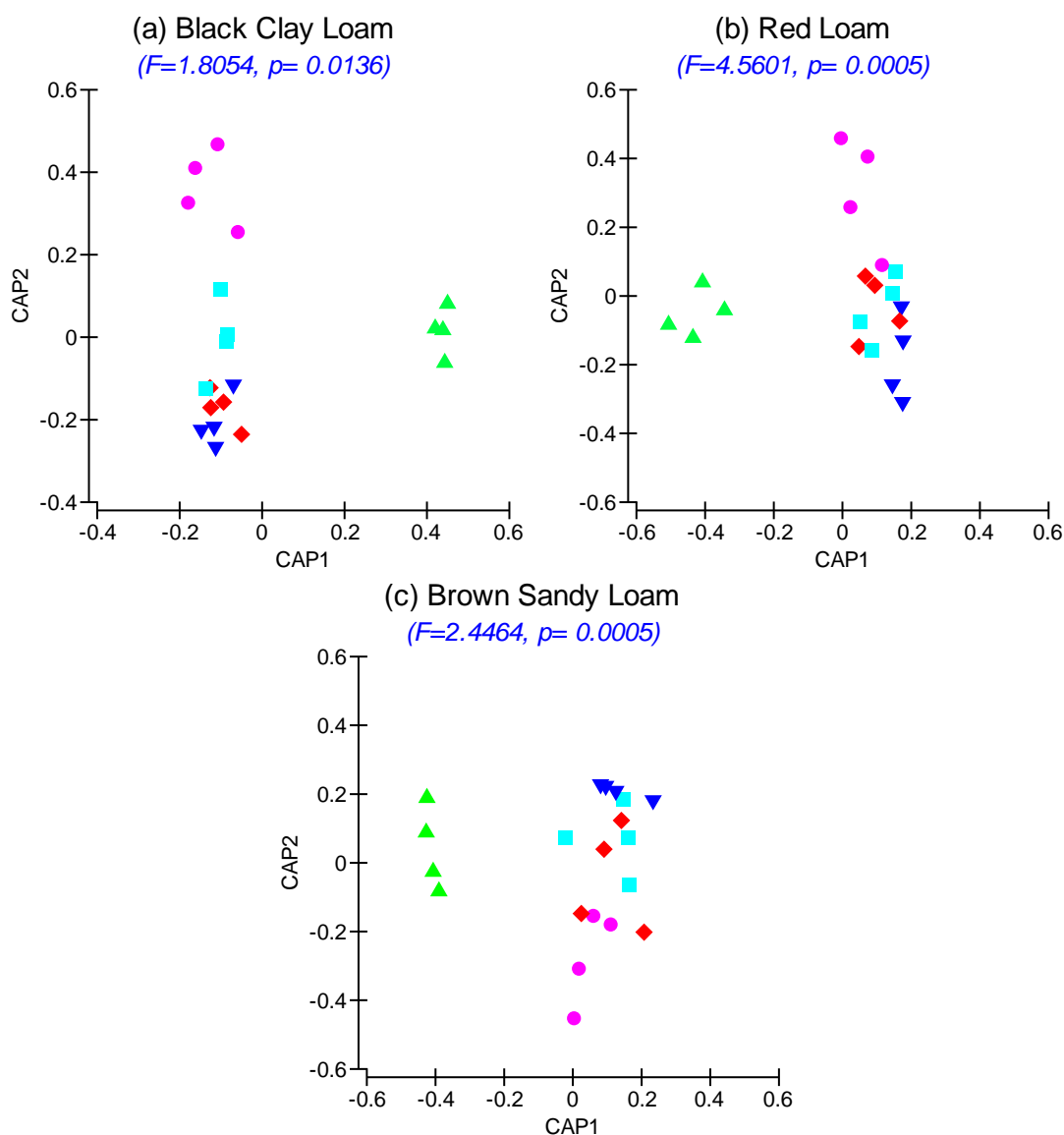


Figure 4.1: Canonical analysis of principal coordinate (CAP) plots showing impact of biochar loading rates on bacterial community structure using 16S rRNA gene sequence analysis data. Comparisons are shown between the initial soil samples, unamended control, 2.5% biochar, 5% biochar and 10% biochar -amended soils. The respective treatment symbols are: ▲ Initial sample, ▼ 0% biochar, ■ 2.5% biochar, ◆ 5% biochar and ● 10% biochar. Each symbol represents an individual soil sample. The assignment of replicates to treatments was assessed using PERMANOVA in each of the soils.

3.3. Bacterial community composition

The dominant bacterial group found at the phylum level was *Proteobacteria* in all soil groups, with proportions ranging between 33- 36% as shown in Fig 4.2. The other dominant bacterial phyla found were *Actinobacteria* (15-22%), *Acidobacteria* (11-14%) and *Verrucomicrobia* (4-13%). No significant differences were found with *Proteobacteria* relative abundance among the biochar treatments in all soils except at the higher biochar level (10%) in BSL soil where it increased in relative abundance by 8-11% compared to the 0% biochar treatment. *Actinobacteria* increased in relative abundance by 51% at 2.5% biochar and 28% at 10% biochar in the RL soil, however decreased in the BCL and BSL soils by 18% at 10% biochar compared to the control. The phylum *Nitrospirae* increased in the BCL soil by 51% and 29% at 2.5% and 10% biochar treatments and also increased by 64% in the BSL soil at 2.5% biochar. However, *Nitrospirae* decreased in the RL soil by 10% and 35% at the higher biochar levels (5% and 10% biochar) compared to the control. The same trend was observed with the *Verrucomicrobia*, which increased by 65% in the BCL at 5% biochar and by 61-63% in BSL at 2.5% and 10% biochar level respectively but decreased in the RL soil by 55-67% in biochar treatments compared to the control. The most dominant class of *Proteobacteria* observed was *Alphaproteobacteria* with an average of 18-21% relative abundance in the BCL soil, 17- 22% in the RL soil and 17- 23% in BSL soils, *Betaproteobacteria* made up 5-7% reads and 4-6% for both *Gammaproteobacteria* and *Deltaproteobacteria*. *Betaproteobacteria* correlated positively with the higher C/N ratio in the BCL soil while a negative correlation was observed in the BSL soil (Table 4.3). No significant correlations were observed in RL soil except for *Deltaproteobacteria* which correlated negatively with the C/N ratio after biochar additions as shown in Table 4.3. Moreover, there was a correlation between the *Alphaproteobacteria* and the increased C/N ratio in the BSL after biochar applications.

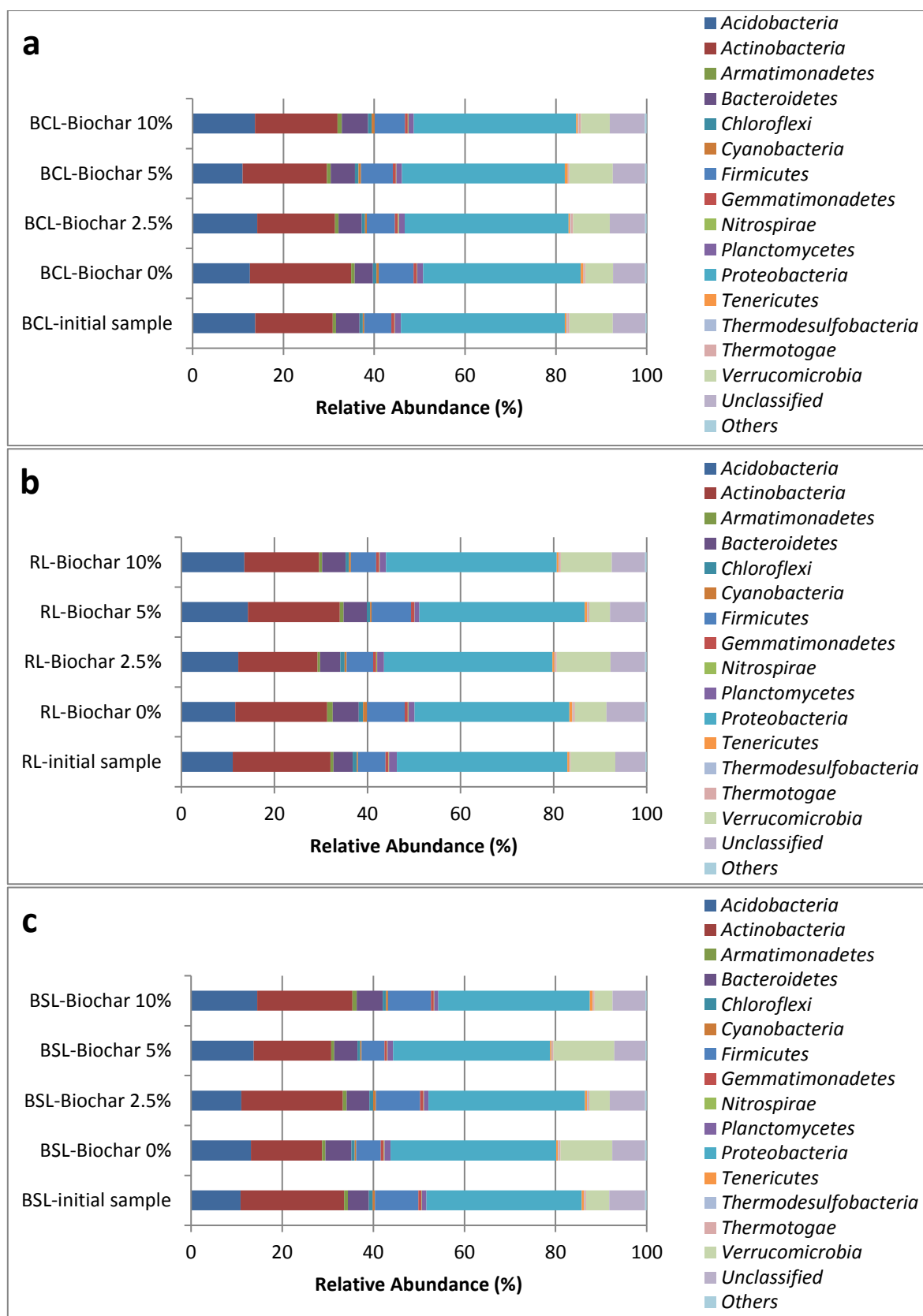


Figure 4.2: Relative abundances of bacterial phyla in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) soils with different biochar amendment rates.

Table 4.3: Correlation of the relative abundances of *Proteobacteria* classes and biochar loading rates (0%, 2.5%, 5% and 10%), and the dominant N₂-fixing bacterial groups (*Azospirillum*, *Bradyrhizobium*, *Rhizobium*, *Frankia* and *Herbaspirillum*) in relation to black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) soil C/N ratios subjected to different biochar loading rates. (*) Indicates significant correlations.

Bacterial group	C/N ratio		
	BCL	RL	BSL
<i>Alphaproteobacteria</i>	-0.37501	-0.63178*	0.85628*
<i>Betaproteobacteria</i>	0.82165*	0.46612	-0.72806*
<i>Gammaproteobacteria</i>	-0.16598	0.55539	0.06865
<i>Deltaproteobacteria</i>	0.02249	-0.93857*	-0.20887

N ₂ -fixing bacterial groups	Biochar loading rates (%)		
	BCL	RL	BSL
<i>Azospirillum</i>	0.7043*	0.2567	0.2967
<i>Bradyrhizobium</i>	0.0036	0.3634	0.3506
<i>Rhizobium</i>	0.9926*	0.1829	0.1848
<i>Frankia</i>	0.0133	0.1943	0.0407
<i>Herbaspirillum</i>	0.0139	0.2902	0.0341

3.4. Ammonia and nitrite oxidizing bacteria

The results showed that in all soils at different loading levels of biochar contained bacterial nitrifiers that were mainly nitrite- or complete ammonia-oxidizing bacteria (Fig. 4.3a), especially *Nitrobacter* and *Nitrospirae*-related groups. No significant differences were found in the proportions of these groups among treatments in RL soil. However, nitrite oxidizing or complete ammonia-oxidizing bacterial relative abundance increased along with the increasing level biochar amendments compared to the control and the initial samples before biochar applications. In particular, *Nitrobacter* in BCL increased by 50% and 53% at 2.5% and 10% biochar levels respectively, whereas *Nitrospira* spp. were found to be 152% more abundant in BCL at 2.5% and 337% and 92% in BSL soil at 2.5% and 10% biochar application rates compared to the control. However, in the BCL soil *Nitrospira* were comparatively more abundant in the initial sample compared to the biochar treatments. On the other hand, the dominant ammonia oxidizing bacteria (AOB) detected included *Nitrosococcus* spp. followed by *Nitrosovibrio* spp. and *Nitrosospira* spp., respectively. In the BCL and RL soils *Nitrosococcus* spp. was higher in abundance in 5% and 10% biochar treatments compared to the control and the initial sample as shown in Fig 4.3a, while the opposite was observed in BSL soil except at higher levels of biochar application. Similarly, the relative abundance of *Nitrosovibrio* spp. increased by 48%, at 5% biochar in BCL soil, by 73% in the RL soil at 2.5% biochar and by 91-93% in the BSL at 5% and 10% biochar rates compared to the control. The proportion of *Nitrosospira* spp. increased in the BCL soil at 5% biochar and in the BSL at 2.5% and 10% biochar while this group decreased in the RL soil with biochar loading rates. Overall, the nitrifying bacteria were mainly affected by biochar applications in the higher N-content BCL and the BSL soils but not in RL soil.

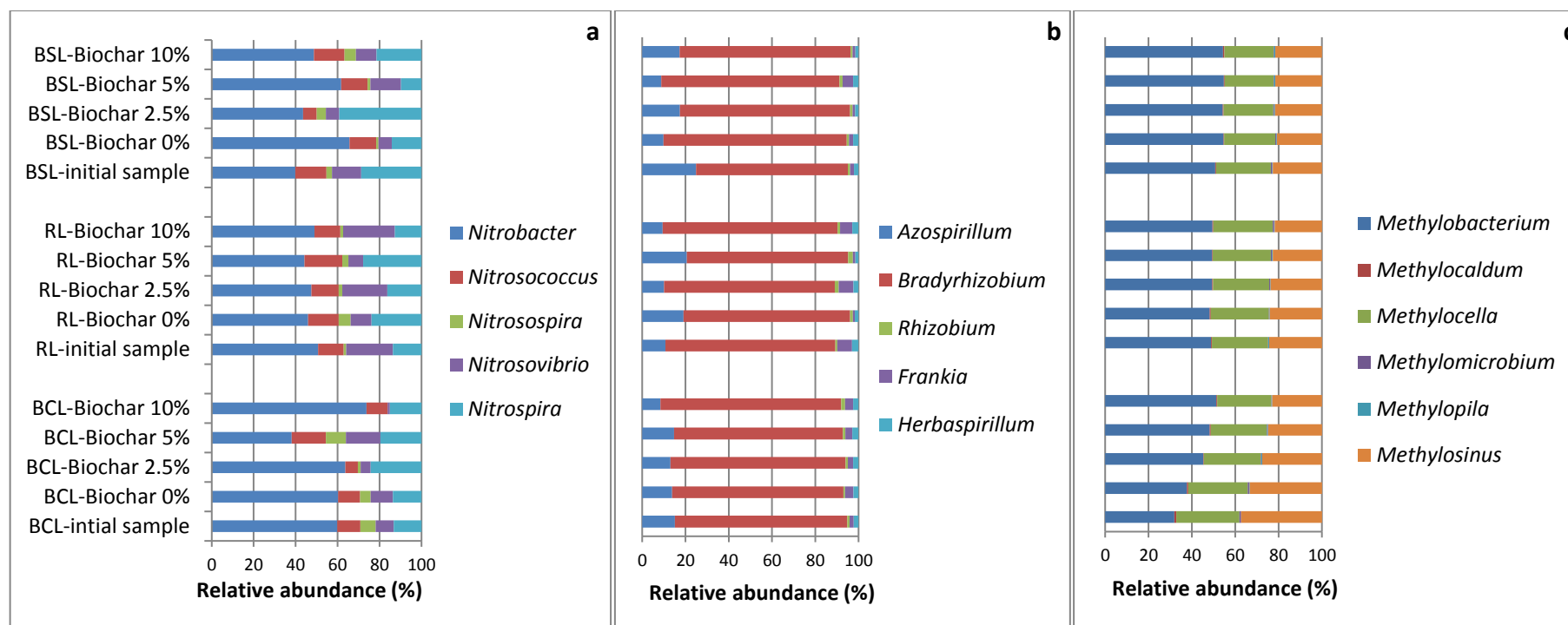


Figure 4.3: Effect of biochar loading rates (0%, 2.5%, 5% and 10%) on the relative abundance of dominant known (a) ammonia and nitrite oxidizing bacteria, (b) nitrogen fixing bacteria and (C) methanol and methane oxidizing bacteria in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) soils. The initial sample is the soil before sieving and biochar addition.

3.5. Nitrogen fixing bacteria

The dominant discernible nitrogen fixing bacterial groups were in order of relative abundance *Bradyrhizobium* spp., *Azospirillum* spp., *Rhizobium* spp., *Frankia* spp. and *Herbaspirillum* spp. in the three soils. The results in Fig 4.3b showed that there was a reduction in *Rhizobium* spp. between the initial sample and biochar treatments in the BCL after 10 months incubation, yet *Rhizobium* correlated significantly ($r=0.99$) with biochar application rates in BCL as shown in Table 3. In the RL soils, there was no difference between the initial sample and the control while *Rhizobium* spp. decreased at 5% biochar levels by 39% compared to the control (Fig. 4.3b). Compared to the control *Rhizobium* increased by 135% at 10% biochar in BCL soil, while in BSL soil *Rhizobium* increased by 96%, 45% and 58% at 2.5%, 5% and 10% biochar levels after 10 months incubation (Fig. 4.3b). *Bradyrhizobium* spp. were the dominant nitrogen fixing bacterial group in all biochar amended soils, no difference was found between the initial sample and the biochar treatments in the BCL as shown in Fig 4.3b. *Bradyrhizobium* spp. was higher in the initial sample in the RL soil (Fig. 4.3b), while no significant difference was found between the other biochar treatments and the control (0% biochar). In the BSL soils, *Bradyrhizobium* spp. was more abundant in the 2.5% and 10% biochar treatments by 50% and 61% respectively compared to the control (Fig. 4.3b). There were only negligible changes in abundance of *Azospirillum* spp. in the BCL (Fig. 4.3b) and RL soils (Fig. 4.3b), however, *Azospirillum* relative abundance correlated positively ($r=0.70$) with biochar loading rates in the BCL soil (Table 4.3). By comparison, in BSL soils the proportion of *Azospirillum* spp. increased by 185% and 202% at 2.5% and 10% biochar treatments while in the control there was no difference from the initial sample following 10 months incubation time (Fig. 4.3b). *Frankia* spp. increased by 379% and 269% with increasing biochar loading rates at 2.5% and 10% in the RL soil (Fig. 4.3b) and by 162% at 5%

biochar treatment in BSL soil but not in BCL compared to the controls (Fig. 4.3b), however the abundance was higher in the initial samples than the amended soils. The abundance of *Herbaspirillum* spp. was higher at 5% biochar rates in the BCL and BSL soils compared to the control while no significant differences were observed in the RL amended soil. The results suggest that biochar addition affects the abundance of N₂-fixers; however the effects are focused on certain groups in a soil dependent manner.

3.6. Methane and methanol oxidizing bacteria

The data indicated that methane and methanol-oxidizing bacterial relative abundance was collectively higher in the BSL soil compared to the BCL and RL soils; however the three soils were dominated by the facultative methylotroph *Methylobacterium*, which was the most affected by the biochar application (Fig. 4.3c). The impact of biochar additions on this genus was higher in the BCL compared to other soils. The relative abundance of *Methylobacterium* increased significantly with increasing biochar loading rates by 54%, 89% and 122% respectively in BCL compared to control. A slight increase was also observed in BSL while no significant differences were observed in the RL after biochar application. Likewise, the type II methanotrophs *Methylocella* and *Methylosinus* increased in relative abundance with increased biochar levels (2.5%, 5% and 10% biochar) in the BCL and BSL compared to the control and the initial samples. *Methylocella* increased by 23%, 39% and 48% in the BCL and 11% in BSL with increasing biochar rates. A similar but less significant increase was observed for *Methylosinus* in BCL and BSL where the abundance increased by 10-20% with increasing biochar application rates. Relative abundance of Methanotrophic bacteria was also higher overall in BCL and BSL biochar amended soils compared to the control and the initial samples except in the RL soil where these groups seemed to be more abundant in the initial sample than the other treatments. The less abundant taxa (*Methylocaldum*, *Methylomicrobium* and *Methylopila*) showed the same response to the biochar additions in the three amended soils. In particular, *Methylomicrobium* increased significantly in the RL by 100%, 150% and 250% among the increased biochar rates as shown in Fig. 4.3c; *Methylopila* increased by 133-167% with increasing biochar rates in the BCL and BSL soils. Overall, biochar additions seemed to cause detectable increases in the proportion of type I and II methanotrophs as well as methanol oxidizing bacteria in BCL and BSL soils but not in RL soils.

4. Discussion

Biochar application has the potential to change the physico-chemical properties which may alter the microbial composition and the related biological processes in soil (Steinbeiss et al., 2009; Lehmann et al., 2011). The result of this study indicated that different application rates of biochar to different soils had a significant impact on NH_4/NO_3 ratios, total C and N, pH, EC and soil moisture content in a short term experiment (Abujabhah et al., 2016b). The impact of biochar varied between soils and at different application rates. Overall, the lighter soils were more affected by the addition of biochar than the heavier clay soil (Uzoma et al., 2011; Abujabhah et al., 2016b). It has been widely stated that biochar may increase the abundance of the microbial community in soil, agreeing with the results observed in this study, especially in BSL amended soil (O'Neill et al., 2009; Abujabhah et al., 2016a). The potential positive impact of biochar addition on soil characteristics and bacterial community structure may reduce $\text{NO}_3\text{-N}$ leaching by reducing nitrification and enhance the biological nitrogen input by increasing immobilisation in soil (Güereña et al., 2015; Xu et al., 2016). A study conducted by Ball et al. (2010) illustrated that soils exposed to fire had a higher abundance of AOB than control soils due to the increased charcoal content. Despite the presence of other AOB, such as archaea in soil, bacteria are more likely to contribute to ammonia oxidation in agricultural soils (Di et al., 2009; Jia and Conrad, 2009). Data from a previous study of Tasmanian soil (Abujabhah et al., 2016a) indicated that archaeal ammonia oxidizers were not substantially influenced by biochar or compost treatments; however this group was not assessed in this study. Our results here instead showed an increase in the abundance of AOB especially in BCL soils with increasing biochar application rates. However, greater changes in the relative abundance of NOB and complete-ammonia-oxidizing bacteria (Daims et al., 2015; van Kessel et al., 2015; Nunes-Alves, 2016) were observed than for AOB. This response may be due to the improvement of physical properties

and pH of BCL soils. Nitrifiers are chemoautotrophs which mean that these bacteria are sensitive to the changes in aeration conditions, nutrient availability and pH caused by biochar application in soils (Banning et al., 2015; Che et al., 2015; Hanan et al., 2016). Many studies indicate that biochar addition affects the abundance and activity of nitrifying bacteria and stimulates potential nitrification rates thus increasing N availability in soil (Berglund et al., 2004; Ball et al., 2010; Prommer et al., 2014; Sorrenti et al., 2017); however, this impact might be diverse, dependent on soil type and biochar application rates (Abujabhah et al., 2016b; He et al., 2016a). Furthermore, recent studies stated that members of the genus *Nitrospira* are capable of carrying out both ammonia and nitrite oxidation in one step referred to as complete nitrification (Daims et al., 2015; van Kessel et al., 2015). Our findings showed that the genus *Nitrospira* was the most abundant and biochar affected group of nitrifiers, especially in the BSL soil, previously shown to have a high nitrification potential (Abujabhah et al., 2016b) , and could be driving changes and availability of nitrogen in the biochar amended soils.

The form of nitrogen in soil is very important in terms of its availability for plant uptake. Since atmospheric N₂ cannot be used, biological nitrogen fixation plays an important role in nitrogen availability, input and retention in agricultural soils. It has been documented that biochar application can improve biological nitrogen fixation rates in soil and enhance the activity of nitrogen fixing bacteria (Rondon et al., 2007; Güereña et al., 2015). However, the mechanism and functionality of biochar impact on biological nitrogen fixation and related microbes is still not clear due to the complexity of the soil ecosystem. Biochar addition may directly affect the growth of specific bacterial groups related to the nitrogen fixation (Beck, 1991a), and also because of the changes in soil properties induced after biochar amendment. Chen et al. (2017) reported that the impact of biochar on microbial population abundance, structure and enzyme activity depended on particle size and application rates, which might be

more noticeable in lighter textured soils, such as the BSL Kurosol studied here. The increased C/N ratio and nutrient availability, especially B and Mo, after biochar application has the potential to improve biological nitrogen fixation in soil (Rondon et al., 2007). The changes in microbial community could be strongly linked to the soil physiochemical properties (Fig. 4) induced by biochar additions (Yao et al., 2017). The findings of this study showed that the relative abundance of nitrogen-fixing microbial taxa differs depending on biochar application rates and soil type; this may be explained by the differences in the initial and amended soil properties following biochar application in different soils. Generally, biochar application seemed to increase the abundance of specific nitrogen related bacteria which correlated to the different soil properties in biochar treatments and soil types (Ducey et al., 2013). The addition of biochar may enhance nitrification especially in heavier soils (BCL) by improving soil properties and affecting nitrifying bacteria which may reduce NH_4 and increase NO_3 in soil (Abujabbah et al., 2016b). The reduction in NH_4 may stimulate symbiosis N_2 -fixing bacteria (*Rhizobia*) to restore the lack of available NH_4 in soil. Moreover, the addition of biochar may improve soil physical characteristics such as aeration which would improve the abundance and activity of free-living diazotrophs such as *Azospirillum*.

Many studies have illustrated that the amount of methane in the atmosphere released as a result of the methanogenic activity in soil is controlled by a combination of microbial and physical processes (Keppler et al., 2009). Biochar potentially reduces the amount of methane released from amended soils (Rondon et al., 2005), possibly due to the reduction of abundance of methanogens by enhanced soil physico-chemical characteristics and increased methane oxidizing bacteria (methanotrophs) in soil. Our results indicated that biochar significantly increased the abundance of methanotrophs with increased application rates, especially in BCL soil. The increased abundance of methanotrophs potentially decreases the amount of methane produced in soil (Feng et al., 2012). The improved soil properties

including pH, aeration and nutrient availability and more importantly the soil moisture content may enhance the CH₄ sink in soil by inhibiting methanogens and/or stimulating methylotrophic activities (Liu et al., 2011; Yu et al., 2013). Methanotrophs could also be using methanol since it was observed facultative methylotrophs such as *Methylobacterium* also become more abundant. Zhang et al. (2010) indicated that soil CH₄ emission correlated with the water content, therefore improved soil physical properties followed biochar additions may contribute to the mitigation of methane emission by improving the structure and aeration in the amended soils.

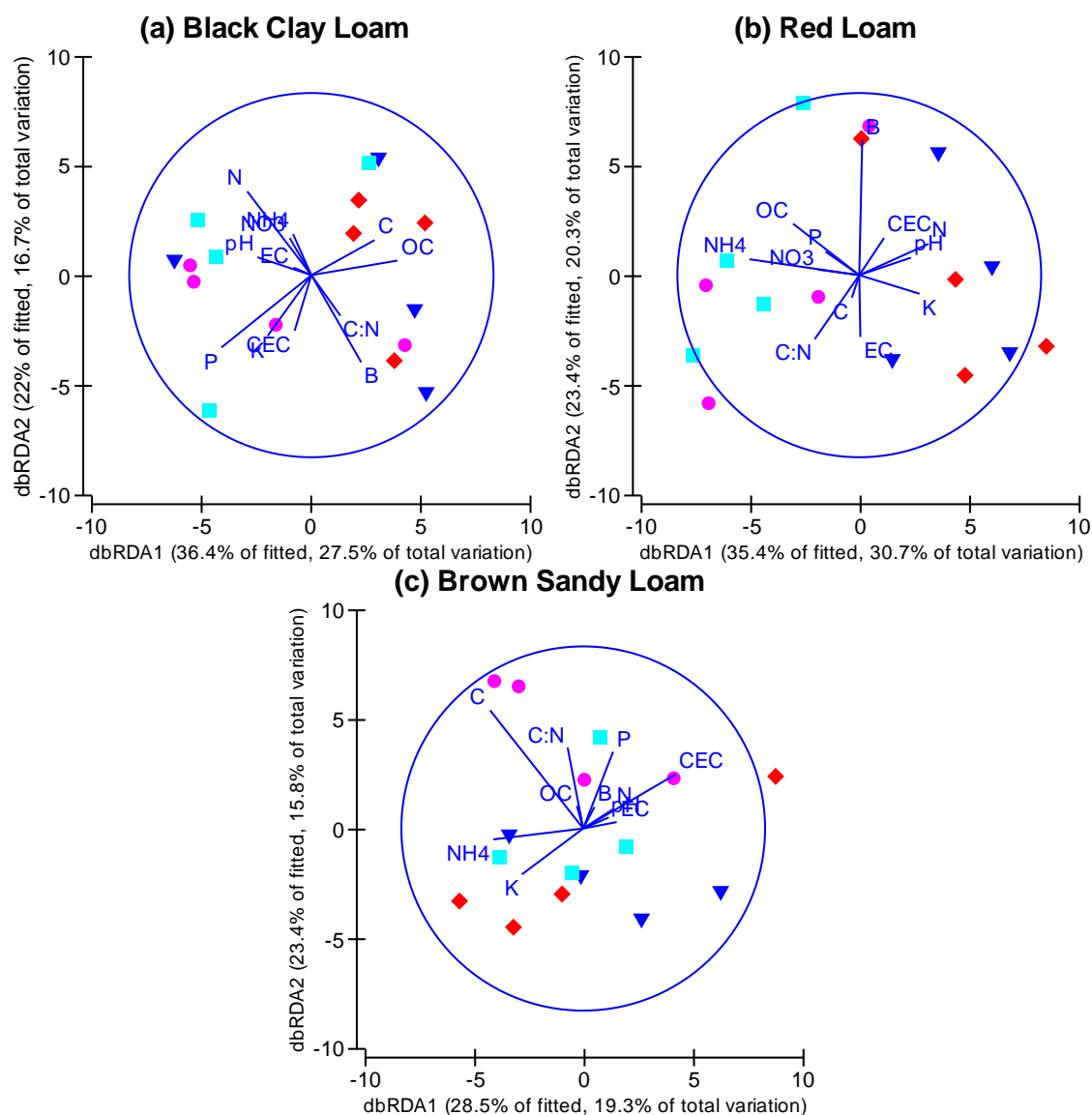


Figure 4.4: Distance-based redundancy analysis (dbRDA) showing the relationship between the significant soil chemical parameters and bacterial community in (a) black clay loam (BCL), (b) red loam (RL) and (c) brown sandy loam (BSL) amended soils. The respective treatment symbols are: ▼ 0% biochar, ■ 2.5% biochar, ◆ 5% biochar and ● 10% biochar. Each symbol represents an individual soil sample. The best fitted and explained variables are shown with vectors with the strength of the correlation indicated by the length of the line (circle donates a correlation of 1.0). The direction of the vector relates the biochar loading level.

5. Conclusion

The results of this study indicated that the application of different rates of biochar significantly enhances soil properties, and yet the impact was dependent on the soil type. Furthermore, biochar addition has greater impact on the bacterial diversity in RL and BSL than the heavier BCL soil. The abundance of nitrifying bacteria increased with increasing biochar rate, especially AOB in BCL soil. However, the abundance of *Nitrospira* and NOB was greater than AOB in all biochar amended soils. Although nitrogen fixing bacteria responded differently to the biochar amendments in different soils, the abundance of selected groups of nitrogen fixing bacteria were increased after biochar additions. Biochar significantly increased the abundance of methanotrophs with increased application rates, especially in BCL soil. This study demonstrates the short term impact of different biochar application rates on bacterial groups that mediate N transformation. Long term studies and measurements of chemical and gas fluxes are required to fully understand the implications of different biochar rates and its interactions in different soil types, including those studied here.

Chapter 5

Eukaryal community changes and composition induced by short term wood-based biochar amendments in three different soils

Abstract

This study determined the loading impacts of wood-based biochar on the eukaryotic community in three different soils (brown sandy loam – BSL, red loam – RL and a black clay loam – BCL) using a pot trial conducted over 10 months. 18S rRNA gene sequencing performed using the Illumina MiSeq platform was carried out to evaluate the changes in eukaryotic community composition in relation to different added amounts of biochar. It was found that biochar addition had a negligible effect on diversity parameters in the brown sandy loam Kurosol (BSL) and red loam Dermosol (RL) soils. There were, however, significant changes in eukaryotic community composition of these biochar amended soils. These changes were most discernible in the lighter BSL soil for the fungal communities ($F=3.0106$, $p=0.0003$) present and also when total eukaryotes were considered ($F=2.3907$, $p=0.0002$). In this respect *Glomeromycota* seem to be slightly promoted in the lighter BSL soils, which might be due to increased soil porosity and soil chemical fertility. Earlier we observed that biochar had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content. The limited impact of biochar loading rates on the soil microbiology could be due to the short incubation period, the lack of added fertiliser nutrients, and also the inherent stability of the soil eukaryotic community. Here we have shown the soil microeukaryotes were affected by short term carbon amendment, though to a limited extent. The data suggested this impact also included important plant symbiotic organisms. Hence the findings

have implications for soil productivity and thus food production in otherwise unfertilised soils.

1. Introduction

Biochar benefits and impacts on biotic processes and microbial diversity have been extensively examined recently, yet the interactions between biochar and the soil microbial community are still unclear. More attention is needed to understand the impact of biochar amendment on soil microbial dynamics and more specifically in relation to nutrient cycling and modifications occurring in biochar amended soils (Tammeorg et al., 2016). Due to its high surface area, biochar can significantly change soil physical properties, especially porosity, and thus could provide habitats for soil microorganisms (Lehmann and Joseph, 2009; Kookana et al., 2011). The use of biochar as a soil amendment may affect physical characteristics and thus soil fertility, which might be different depending on pyrolysis conditions used for creating the biochar. The variation in physical properties between biochar and the soil matrix leads to an overall change in soil density and aggregation, hydraulic conductivity and gas transportation, which in turn impacts chemical properties and presumably the subsequent composition and activity of soil microorganisms (Lehmann et al., 2011).

Biochar application could also improve water management and enhance fertiliser treatment response in soil. A experiment conducted by Asai et al. (2009) to investigate the effect of biochar amendment on soil physical properties and rice green yields within upland conditions in ten sites at application rates from 0-16 t h⁻¹, were combined with N and P fertilizer application rates. The results showed an improvement in hydraulic conductivity and other physical characteristics in soil, which provides suitable conditions for microorganisms. The

impact of biochar addition in soil could persist for extended periods of time due to high resistance to microbial degradation. The various contributions and effects of biochar application in different soils requires more clarification which could assist in the choice of particular biochar for specific soils in order to gain the maximum benefits from biochar as a soil amendment (Sohi et al., 2010).

The interactions between different biochars and soil types are complicated due to the variations in biochar feedstocks and soil chemical properties as demonstrated by Unger et al. (2011). Biochar has also been shown to increase soil pH, cation exchange capacity (CEC) and thus nutrient holding capacity, influencing nutrient concentration in soils and their availability for crop uptake (Fowles, 2007). Anion exchangeable capacity (AEC) can also be enhanced, holding plant and microbe available nutrient anions such as phosphate and sulphate (Chan and Xu, 2009; Atkinson et al., 2010; Lawrinenko and Laird, 2015).

The high surface area of biochar is likely to provide ideal colonisation sites for soil microorganisms due to the high concentrations of adsorbed elements and organic substances, including nutrients. Many reports indicate that biochar amendment enhances microbial populations and activity in soil by inducing nutrient availability, metabolism and growth of soil microorganisms (Kookana et al., 2011; Tong et al., 2014). The changes that biochar can cause through increasing organic matter content, pH, total nitrogen and phosphorus, exchangeable cations and CEC would be the most logical reason for the enhancement of microbial populations and activity (Kelly et al., 2014). However, the specific changes, behaviour and adaptation of soil microorganisms associated with particular kinds of biochar in different soils still need to be considered (Chan et al., 2008; Lehmann et al., 2011). Previous studies indicate that biochar creates a suitable environment for microorganisms, enhancing population growth and microbial abundance in soil, but there is a variation in the

bacterial and fungal ratios because of the increase in C/N ratio and soil pH after biochar addition (Kookana et al., 2011; Chen et al., 2013).

The purpose of this study was to determine the loading impact of wood-based biochar on the eukaryotic community in Kurosol, Dermosol and Vertosol topsoils. The experiments were performed in a minimally managed, non-fertilized pot trial to measure the effects of biochar on the soil community after a period of 10 months. Biochar from green waste was mixed with the topsoils at four loading levels from 0 to 10% w/w (Abujabhah et al., 2016b). Previous results from the modelled system showed that biochar additions increase soil carbon and pH, although the effects varied with soil types. Biochar loading rates also significantly affected NH_4 and NO_3 , N, EC and soil moisture content, yet the impact was different with each soil type. Significant impact was observed on potential nitrification, carbon utilisation and microbial activity at higher biochar loading rates in lighter soils. The increase of total C after biochar application will increase labile carbon decomposition in soil which increases N utilisation by microorganisms. To assess eukaryotes in the soils studied a next-generation sequencing approach was used in which the 18S rRNA gene data were obtained and analysed to compare eukaryotic community composition. These analyses were conducted in both the original field soils and in biochar treated and incubated soils. The objectives were to determine if biochar addition systematically affected the eukaryotic community structure in the absence of other variables, such as fertilizer input, within a short time period. The second goal was to determine whether soil type was influential in influencing the impact of the biochar. Finally the effect of loading of biochar was evaluated to determine the sensitivity of the eukaryotic community to the amount of carbon amendment.

2. Materials and methods

2.1. Soil Collection and Biochar Specifications

Three different topsoils were collected for use in this experiment. A black clay loam (BCL) Vertosol was collected from a forested hillside located near the Horticultural Research Centre, University of Tasmania, Hobart, Tasmania (42° 54. 25.92' S 147° 19.22.35 'E). A loamy red (RL) Dermosol was collected from a farm near Cambridge, Tasmania (42° 48.11.77'S 147° 26.22.03'E). Brown sandy loam (BSL) Kurosol was collected from an uncropped site in an apple orchard at Mountain River, located in the Huon Valley region of south eastern Tasmania (42°57'2.91"S, 147°5'52.13"E). Soil subsamples were kept frozen for biological analysis; the remaining soils were mixed with biochar and used for the pot experiments after all the plant residues and gravel material were removed. Biochar used in this experiment was sourced from eucalypt green waste and produced in an updraft rotary hearth gasifier operating with a peak temperature of 650 - 750 °C (feedstock dependant), with oxygen limited atmosphere and residence times not longer than 3 minutes (most typically around 100 seconds).

2.2. Experiment layout

Biochar was mixed through the three different soils at four different rates (% , 2.5%, 5%, 10% w/w). The prepared mixes were placed into 1.5 L pots and left exposed to the natural elements for 10 months. The annual rainfall during the experiment was 613.3 mm which the mean maximum and minimum temperatures were 19.9°C and 8.3°C respectively (Ellerslie Road Weather Station, Lower Derwent, Tasmania). The pots were not planted or fertilised to limit other factors that may obscure the impact of biochar addition on soil properties and

microbial community. However, natural volunteer weed vegetation grew during the experiment most strongly on the RL and BSL soils. Each biochar-soil treatment was replicated four times and arranged randomly in a grid of 48 pots.

2.3. Soil sampling and chemical analysis

Soil samples were taken from the pots after 10 months incubation for instant water content measurements and chemical analysis. Selected soil chemical parameters were analysed by CSBP Laboratories, Western Australia to estimate the potential changes in the three soils after biochar amendments. These results are reported previously in Abujabhah et al. (2016b).

2.4. Scanning Electron microscopy analysis

Samples from the amended soils and the original biochar used in this experiment were collected, coated with atomised gold and analysed using scanning electron microscopy (Hitachi SU-70 field emission scanning electron microscope -FESEM) to observe the pore morphology and surface properties of biochar after 10 months incubation time in soil. Also uncoated samples from biochar amended soils were investigated via environmental scanning electron microscopy (FEI MLA650 environmental scanning electron microscope - ESEM) analysis.

2.5. DNA extraction and soil biomass assessment

DNA was extracted from the soil samples using PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc) by following the provided protocol. Soil biomass was estimated as bacterial counts and DNA quantity. Extracted DNA (n=4 replicates for each treatment) was checked for quality at 260/280 nm and quantified via Nanodrop spectrophotometer (NanoDrop 8000 Spectrophotometer, Thermo Fisher Scientific Inc., Wilmington, DE 19810 U.S.A.) to estimate soil biomass (Marstorp et al., 2000; Bouzaiane et al., 2007). Bacterial enumerations were estimated as colony forming units (CFUs) on 10% Tryptone soy solid agar plates incubated at 25°C for 21 days (Juhnke et al., 1987).

2.6. 18S rRNA sequencing and bioinformatics analysis

Sequence analysis was performed at the Ramaciotti Centre for Genomics (Kensington, NSW, Australia) where the V9 region of 18S rRNA genes were amplified using 12 bp tagged universal primers 1391F (TATCGCCGTT CG GTACACACCGCCCGTC) -EukBr (AGTCAGTCAG CA TGATCCTTCTGCAGGTTTCACCTAC) (Amaral-Zettler et al., 2009; Caporaso et al., 2012; Hugerth et al., 2014), respectively. The primers have high coverage read > 80% of eukaryote sequences (Hugerth et al., 2014) and the V9 region is shown the best informative mechanism for biodiversity analysis. Sequencing was performed using the Illumina MiSeq platform according to standard protocols generating 300 bp pair-ended reads. All reads were filtered based on quality scoring, trimmed of the tag regions, chimera checked using QIIME. The 18S rRNA paired-end FASTQ reads were joined into one single read and then converted into FASTA files using Galaxy (<http://galaxycast.org>) as described by Blankenberg et al. (2010). The sequences were also uploaded in the MG-RAST server

(<http://metagenomics.nmpdr.org>) (project id: 12994) for annotation, classification and metagenomics analysis (Meyer et al., 2008). The abundance data were extracted at the best hit classification using the default MG-RAST setting where the data was compared to the M5NR database (Wilke et al., 2012) with annotation utilizing maximum e-value of $1e^{-5}$, a minimum identity of 60 %, and 15 as a minimum alignment length cut-off. The 18S rRNA paired-end FASTQ reads were also filtered and joined using Mothur (http://www.mothur.org/wiki/MiSeq_SOP) following the protocol described by Kozich et al. (2013). The produced reads were then uploaded to SILVAngs (<https://www.arb-silva.de/ngs/>) where all reads were processed by the NGS analysis pipeline of the SILVA rRNA gene database project (SILVAngs 1.3) (Quast et al., 2013). Each read was aligned using the SILVA Incremental Aligner (SINA SINA v1.2.10 for ARB SVN (revision 21008) (Pruesse et al., 2012) against the SILVA SSU rRNA SEED and quality controlled (Quast et al., 2013). Reads shorter than 50 aligned nucleotides and reads with more than 2% of ambiguities, or 2% of homopolymers, respectively, were excluded. Putative contaminating sequences, artefacts and reads with a low alignment quality (50 alignment identity, 40 alignment score reported by SINA), were identified and excluded from downstream analysis. After these initial steps of quality control, identical reads were de-replicated with unique reads clustered (OTUs), on a per sample basis, and the centroid reference read of each OTU subsequently classified. De-replication and clustering was done using cd-hit-est (version 3.1.2; <http://www.bioinformatics.org/cd-hit>) (Li and Godzik, 2006) running in accurate mode, ignoring overhangs, and applying identity criteria of 1.00 and 0.98, respectively. The classification was performed by a local nucleotide BLAST search against the non-redundant version of the SILVA SSU Ref dataset (release 123.1; <http://www.arb-silva.de>) using BLASTn (version 2.2.30+; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) with standard settings (Camacho et al., 2009). The classification of each OTU reference read was mapped onto all

reads that were assigned to the respective OTU. This yields quantitative information (number of individual reads per taxonomic path), within the limitations of PCR and sequencing technique biases, as well as, multiple rRNA operons. Reads without any BLAST hits or reads with weak BLAST hits, where the function “($\% \text{ sequence identity} + \% \text{ alignment coverage}$)/2” did not exceed the value of 93, remain unclassified. These reads were assigned to the meta group (No Relative) in the SILVAngs fingerprint and Krona charts (Ondov et al., 2011).

2.7. Statistical analysis

Soil chemical parameters were statistically tested using SPSS v22 to assess the effect of biochar additions on soil properties compared to unamended control treatments. Permutation multivariate analysis of variance (PERMANOVA) (Anderson et al., 2005), and canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003) were conducted using default settings with 9999 permutations, while CAP was conducted using default settings to assess the microbial community compositions. For sequence read analysis, the data was organised at the lowest taxonomic level possible and normalised as percentages, square root transformed and a resemblance matrix created by calculation of Bray-Curtis coefficients. The PERMANOVA derived significance values were considered significant when $P < 0.01$, while $0.01 < P < 0.05$ were considered only marginally significant. PRIMER-6 was used to calculate Shannon (H' , log base e), Pielou's evenness (J'), and Fisher's α -diversity. Good's coverage was calculated as $1 - (n/N) \times 100$, where n is the number of singleton OTUs and N is the total number of sequences in the sample. The linear discriminant analysis effect size (LEfSe) method was used to estimate the significant differences in the eukaryotic taxa between the amended soils (Segata et al., 2011).

3. Results

3.1. Soil physicochemical properties

Previous soil analysis results reported by Abujabhah et al. (2016b) indicated that the addition of different levels of biochar to different soils had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content. No significant impact was observed on extractable P or K (Colwell method), exchangeable cations or CEC in BCL soil with the exception of exchangeable K. However in both BSL and RL soils, biochar addition had significant effects on exchangeable Al, Ca, Mg and Na. The main impact among biochar treatments across all three soils was that increasing loading increased the C/N ratio and soil pH as described in more detail by Abujabhah et al. (2016b).

3.2. Electron-microscopic analysis of biochar

Scanning electron-microscopic analysis (SEM and ESEM) was used to visualise the pore sizes and the interaction with soil particles in the biochar within the amended soils and the original biochar used in this experiment. The images showed that the biochar had a wide range of pore sizes, ranging two orders of magnitude (1 – 100 μm). The micrographs show that biochar pore sizes vary significantly with the dominant size-cluster of approximately 5 – 20 μm and minor size-cluster at 40 – 60 μm . These finer pores lie in the ‘plant available water’ (PAW) range. The biochar also maintained much of this porous structure for over the 10 months period although with noticeable breakage and associated pore collapse and minor in-fill with soil as compared to the fresh biochar (Fig. 5.1). The incubation time and experiment preparation might have contributed to the heterogeneity of the surface structure and pore size distribution.

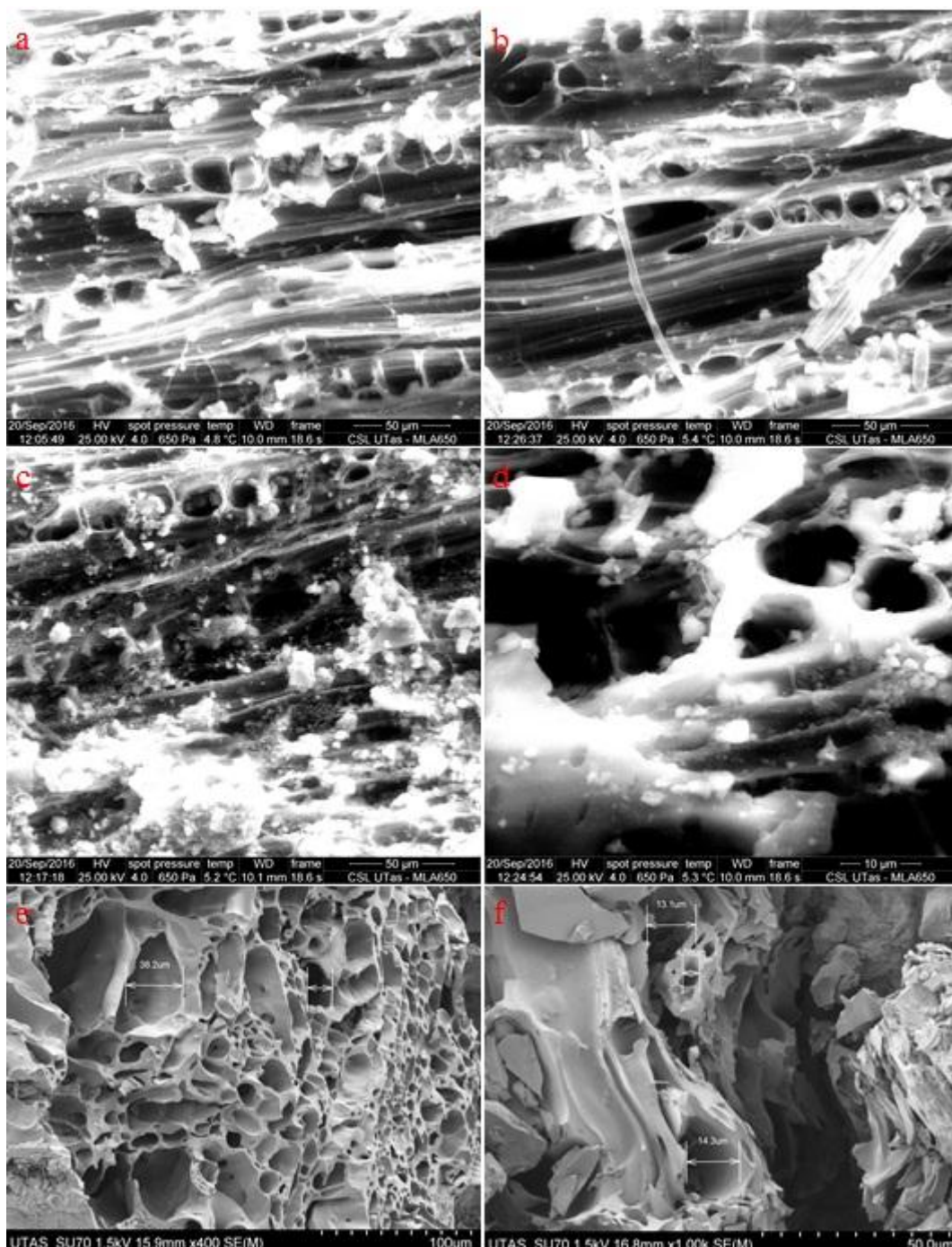


Figure 5.1: Microscopic images from ESEM (a-d) of biochar amended soils and SEM of biochar separately (e) and biochar mixed with soil (f) at different magnifications.

3.3. Eukaryotic alpha diversity

A total of 11,181,813 sequence 18S rRNA reads were obtained from all the soil samples with an average of 186,364 reads per sample. As shown in Figure (5.2) the lighter topsoils (RL and BSL) were more even (0.65 – 0.68 versus 0.53) and proportionally more diverse (2.86 – 2.94 versus 2.32, based on the Shannon index) than the heavier Vertosol BCL topsoil, otherwise the average number of OTUs detected were similar, ranging from 15,000 – 23,000 (average 18,864). BSL had the greatest species richness (average 10.1) compared to BCL and RL soils (9.12-9.21). The species richness in the RL topsoil was impacted by the preparation of the pots as the initial samples had lower species richness (9.21) compared to the homogenised and 10-month treated zero biochar soil (10.30).

The addition of biochar overall did not have any profound effect on eukaryotic soil diversity after 10 months, and effects that did occur were soil dependent. The number of OTUs increased in the BCL and BSL amended soils compared to the initial samples and the control biochar soil by 8-25%, while the opposite occurred in the RL amended soils (reduced 6-20%) (Fig. 2). Moderate loadings of biochar seem to achieve maximal OTU number increases (2.5% for BSL and 5% for BCL), while for RL soil the lowest OTU number was recorded at 10% loading, though there was no consistent trend associated with loading levels.

In terms of species richness, only biochar amendment in the BCL topsoil showed a discernible effect, with richness increasing from 9.3 to 9.55 – 9.85 in the amended soil (highest with 2.5% biochar). In terms of microbial species evenness biochar amendment had no significant effect (Fig. 5.2). Biochar amendments in BCL soils resulted in an increase in Shannon diversity (from 2.59 to 2.61 – 2.85) peaking with 10% biochar loading (2.85), though biochar loading level itself was not significant in specifically affecting diversity.

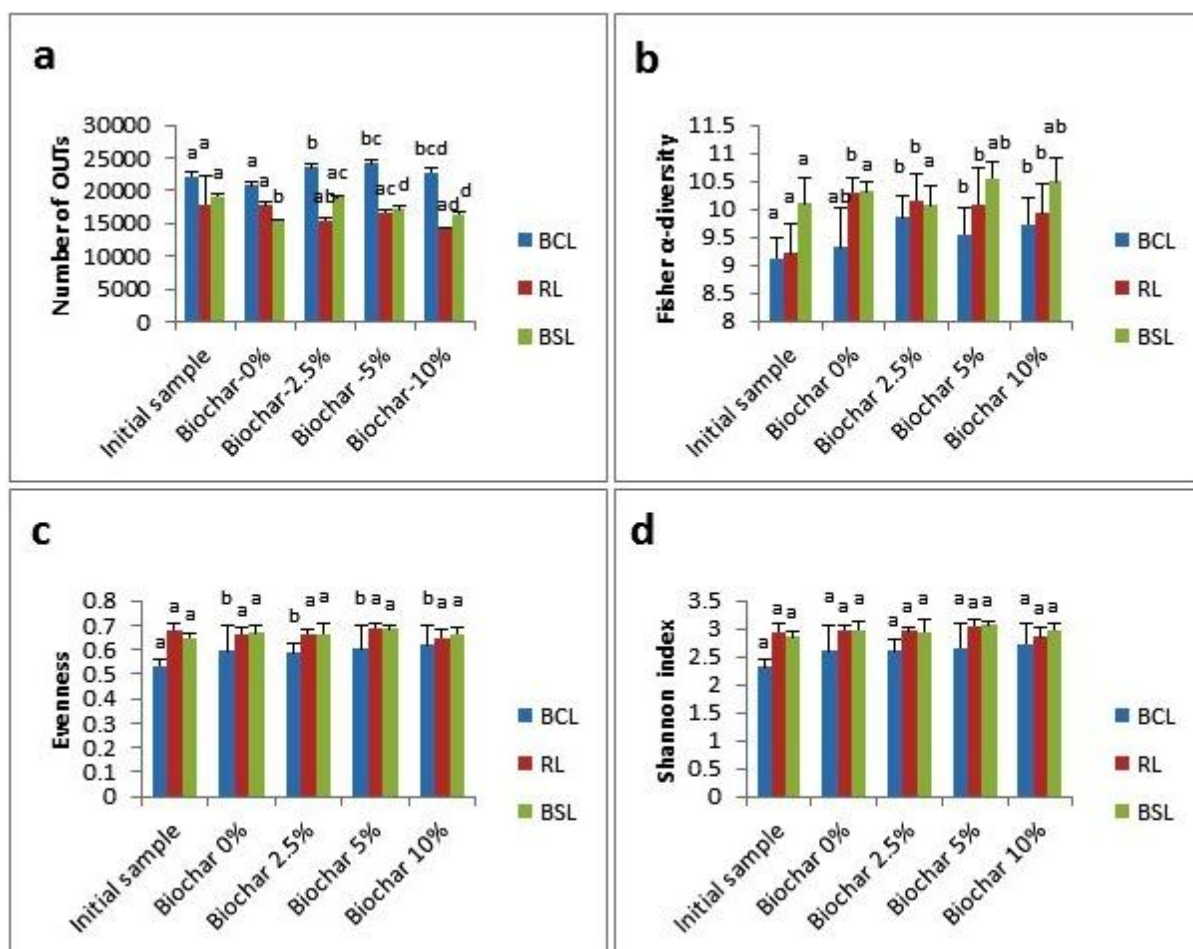


Figure 5.2: (a) the number of OTUs, (b) Fisher α -diversity (c) Evenness and (d) Shannon index in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) soils in the initial samples and biochar treatment. Error bars present standard deviation and letters indicate significant differences between treatments among amended soils.

The rarefaction curves as shown in Figure (5.3) indicated that none of the samples reached an asymptote, with Good' coverage estimations of 84%, 82% and 83% for BCL, RL and BSL respectively. Based on the OTU versus sequence totals, biochar amendment in the BCL and BSL soils resulted in greater OTU numbers as also indicated above; however the different biochar rates all gave similar results in the case of the BCL soils, while in the BSL soil 2.5% w/v amendment resulted in greatest OTU numbers. Overall, only the BCL soil showed consistent responses to biochar loading and even then the level of loading did not have any specific effect on diversity parameters.

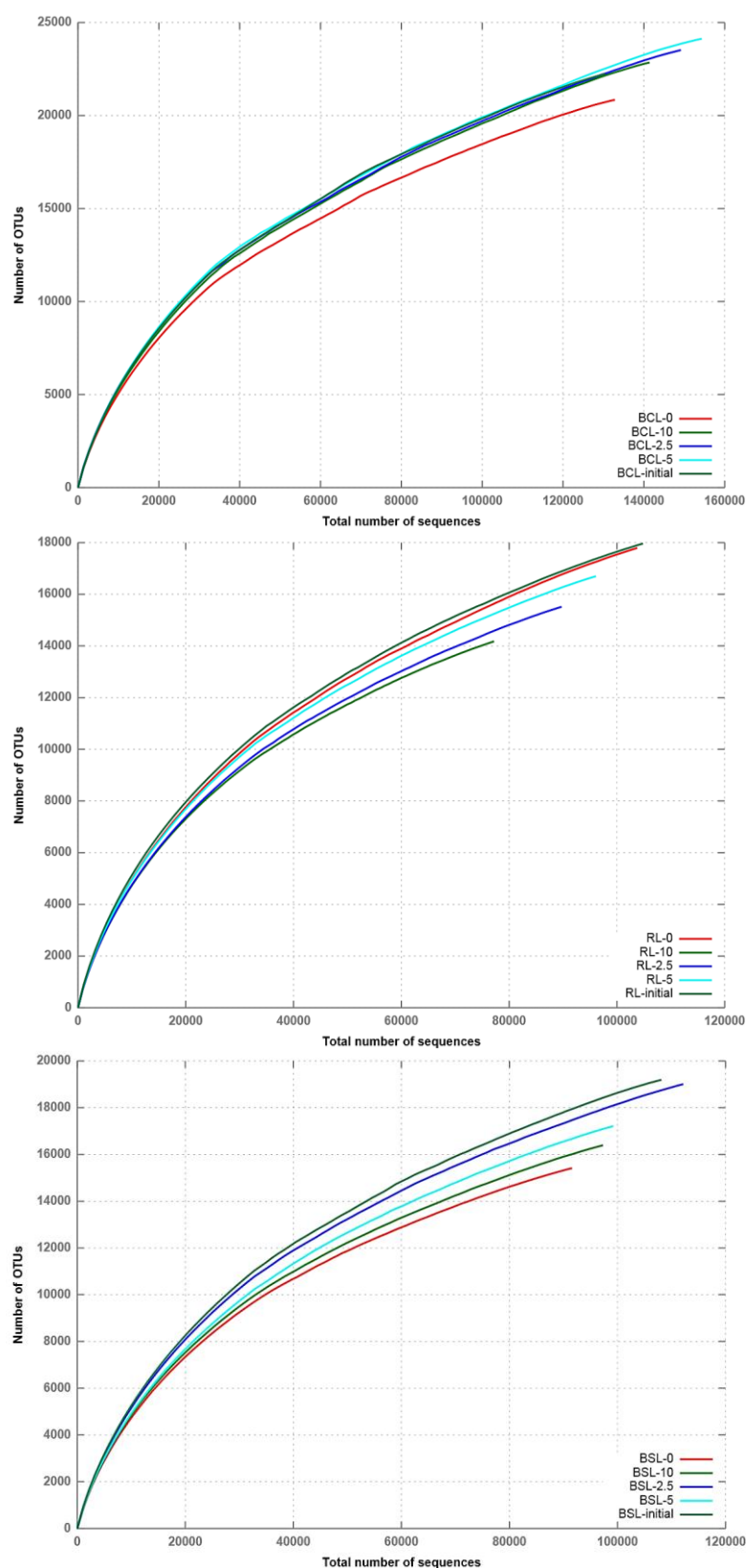


Figure 5.3: Rarefaction curves for total eukaryote community in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) in the initial samples and biochar treatments.

3.4. Multivariate analysis of eukaryotic populations

Setting up the pot experiment and incubating for 10 months resulted in a significant change in both fungal and overall eukaryotic communities. This difference would also incorporate the homogenising effect caused by the physical biochar addition. PERMANOVA analysis otherwise indicated that biochar additions had a marginal ($p \geq 0.01$) to moderate impact ($p \geq 0.001$) on the fungal ($F=1.3728$, $p=0.0317$) and total eukaryote ($F=1.5127$, $p=0.0034$) communities in the RL, ($F=0.8673$, $p=0.6541$) and ($F=1.333$, $p=0.0599$) in BCL soils, respectively. The impact of biochar additions was more discernible on the fungal ($F=3.0106$, $p=0.0003$) and total eukaryote ($F=2.3907$, $p=0.0002$) community in the lighter BSL soil (Fig. 5.4). Overall, biochar loading treatments had little separation in the BCL soil CAP plots while sample groups were better classified in the case of RL and BSL soils.

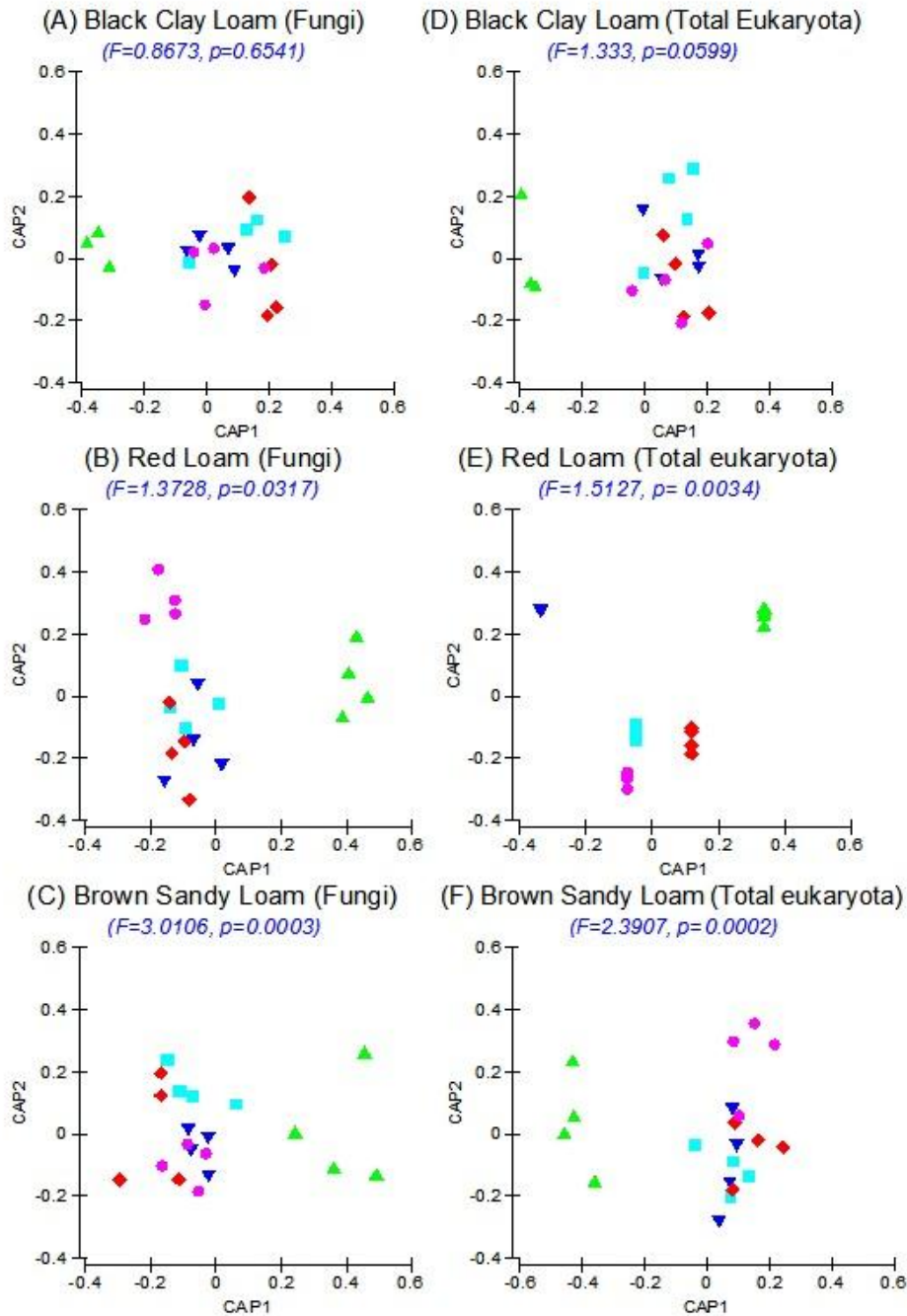


Figure 5.4: Canonical analysis of principal coordinate (CAP) plots of fungi and total eukaryote community structure determined from taxa classifications derived from 18S rRNA gene sequence analysis data. Comparisons are shown between initial sample before conducting the experiment, unamended control, 2.5% biochar, 5% biochar and 10% biochar amended soils. The respective treatment symbols are: ▲ Initial sample, ▼ 0% biochar, ■ 2.5% biochar, ◆ 5% biochar and ● 10% biochar. Each symbol represents an individual soil sample. The classification of replicate data treatment was assessed using PERMANOVA in black clay loam, red loam and brown sandy loam soils.

3.5. Fungal community composition and the effect of biochar loading

The three soils were dominated by the phylum *Ascomycota* with class *Eurotiomycetes* (asci-forming fungi, 17.5-46.3% of reads) contributing the greatest proportion of reads at the class level, especially in BCL soil as shown by the LEfSe analysis (Fig. 5.7). Other major groups of fungi that were well represented in soils included *Agaricomycetes* (“mushroom”-forming fungi, 14.4 – 28.5 % of reads), *Dothideomycetes* (bitunicate asci-forming fungi, 3.0-15.4%), *Saccharomycetes* (budding yeasts, 1.0 – 6.9%), *Sodariomycetes* (fungi that form asci in perithecial fruiting bodies, 1.3 – 6.3%) and *Tremellomycetes* (“jelly” fungi, 1.6 – 3.5%). A large proportion of reads (20.5 – 32.3%) could not be classified to class level (Fig. 5.5a).

In the initial BCL soil samples, class *Eurotiomycetes* were very abundant (46.3% of reads) (Fig. 5.5a). The impact of biochar loading was minimal in the BCL soils as suggested by the CAP/PERMANOVA analysis. The experimental set-up and the 10 month pot trial itself generally had only a minor effect. The main differences were a greater abundance of *Neocallimastigomycetes* (anaerobic plant fibre-degrading fungi) and *Leotiomycetes* (fungi that mostly have asci in apothecia) and lower levels of *Sodariomycetes* when initial samples were compared with the 0% biochar treatment soils. The relative abundance of *Eurotiomycetes* ranged between 34-39% among biochar treatments in BCL soil, only slightly lower than 0% control (41%). Similar level variations (<2 fold) in relative abundances occur for most other fungal taxa. An increase in *Sodariomycetes* was observed with 1.1% of reads in the control, increasing to 2.3-6.0% reads in the biochar amended samples (6.0% in the 10% w/w loaded samples similar to the initial sample). A progressive reduction in *Leotiomycetes* abundance occurred with loading from 3.4% of reads in the control to 0.8% in the 10% loaded samples. Loading of biochar also seemed to reduce the relative abundance of lichen

fungi of class *Lecanoromycetes* (from 0.9% to 0.1-0.2%). Overall, the data suggests biochar loading has minimal effects in BCL soils though it may promote *Sodariomycetes*.

In the initial RL soils *Dothideomycetes* and *Wallemiomycetes* (xerophilic moulds) were relatively abundant compared to the other soil types. Setting up the experimental pot system strongly promoted *Neocallimastigomycetes* and reduced the *Dothideomycetes* relative abundance. Weaker stimulation of *Orbiliomycetes* (saprobic sac and nematode trapping fungi), *Paraglomeromycetes* (arbuscular mycorrhiza, (Oehl et al., 2011)) and *Saccharomycetes* abundance also occurred. The effect of biochar loading, like the BCL soil had minimal consistent effects on most class-level fungal taxa, variations likely mostly reflect patchiness within the samples. The 10% biochar loaded sample, which would be expected to have the greatest physio-chemical alterations stood out in that samples contained *Exobasidiomycetes* (plant parasitic fungi, 4.2% of reads on average), *Schizosaccharomycetes* (fission yeasts, 5.3%), *Taphrinomycetes* (plant parasitic fungi, 1.2%), and higher abundance of *Tremellomycetes* (3.9% versus 0.9-1.4%). Overall, RL soils were not strongly affected by biochar except at the highest loading level.

In the BSL soils setting up the pot trial resulted in effects analogous to what was observed with the RL soils. *Neocallimastigomycetes* and *Saccharomycetes* were promoted in relative abundance. Other taxa stimulated included the *Archaeosporomycetes* (a type of arbuscular mycorrhiza, Oehl et al. 2011), *Hyphochytriomycetes* (stramenopiles formerly fungi) from the LEfSE analysis (Fig. 5.8). The effect of biochar loading itself was much less evident. *Glomeromycetes* became more abundant in biochar amended soil (0.8-3.1%). *Lecanoromycetes* also become less abundant (1.4% dropping to <0.1%), as observed for BCL soils. Overall, biochar showed little evidence of impacting BSL soil fungal communities.

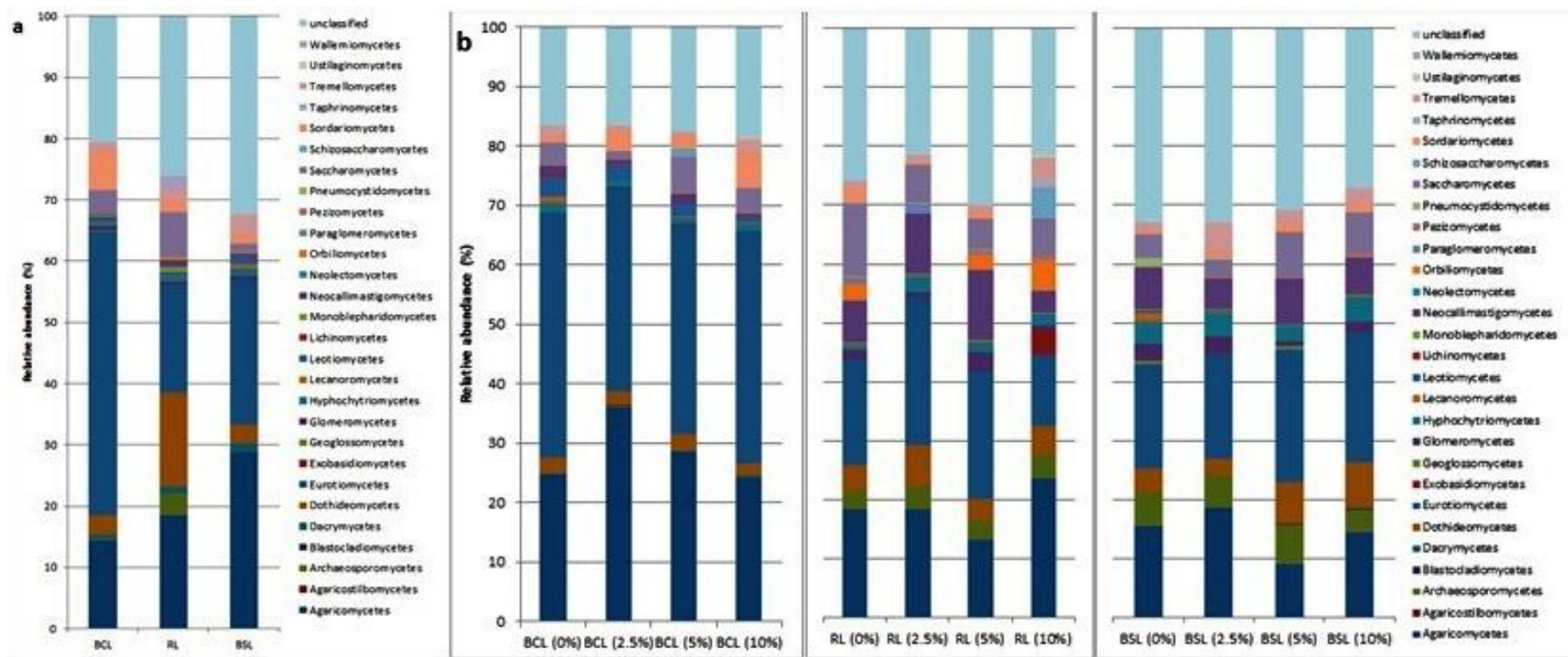


Figure 5.5: Abundance of fungal groups at the class level in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) in the (a) initial samples and (b) biochar treatment.

3.6. Composition of soil metazoa and other eukarya and the effect of biochar addition

The proportions of 18S rRNA sequences derived from metazoan inhabitants of the test soils are shown in Figure 6. Initial soil profiles show differences that reflect the nature of the soil types used in the experiment. Most metazoa in the BCL soils were nematodes (*Chromadorea*, *Enoplea*) and annelid worms (*Polychaeta*) making up 87.8% of reads. In RL soils this proportion was only 33.6%, instead this soil had a much higher content of *Eutardigrada* (24.6% of reads), *Trematoda* (4.9%), *Insecta* (12.2%), and *Nassophorea* (9.0%) (ciliate protists). BSL soils had 37.3% of reads derived from nematode and annelid taxa. The other major community members include *Insecta* (26.0%), *Gastropoda* (7.1%) and *Nassophorea* (25.5%) (Fig. 5.6).

Setting up the biochar experiment resulted in substantial changes to the BCL soil with *Chromadorea* dramatically reduced (47.3% to 2.1-8.1% of reads) while increases occurred with *Insecta* and *Arachnida*. The effect of biochar was minimal on the BCL topsoils though this was not helped by a large proportion of unclassified taxa (20-35% of reads) and the quite variable distributions between soil, reflecting patchy distributions of metazoa in the sample replicates analysed. Biochar loading rate may have stimulated *Gastropoda* abundance (2.5-15.0% versus 1.7% of reads).

In the case of the RL soil, the establishment of the experiment resulted in mainly loss of *Eutardigrada* (24.6 to 2.0% of reads) and a large increase in *Nassophorea* relative abundance (9.1 to 42.0%). The effect of biochar loading was substantially less obvious. *Chromadorea* appeared to be slightly more abundant in biochar amended samples compared to the 0% control (15.9-17.9% versus 10.8% of reads) while annelids were least abundant in the 10% biochar amended soils (3.5% of reads versus 6.4-10.9% in the other samples and control).

BSL soil metazoan composition was also affected by experimental set-up. *Chromadore* (38.2% in the 0% control versus 6.3% of sequences in the initial samples), *Eutardigrada* (9.7% versus 0.4%) became more abundant while *Polychaeta* was less abundant (1.9% versus 9.0%). The effect of biochar was only evident for *Polychaeta* where more sequences of this group were detected in the biochar containing samples (9.4-16.0% of reads compared to 1.9% in the control). The 5% w/v biochar had a large proportion of *Malacostraca* (crustacean), mainly isopods, not detected in the other samples.

Overall, the effect of biochar loading was relatively minimal as suggested by the spatial distributions in the CAP/PERMANOVA plots (Fig. 5.4). The main changes that occurred seem to affect taxa in the RL and BSL soils to a greater extent; however the taxa affected was soil dependent

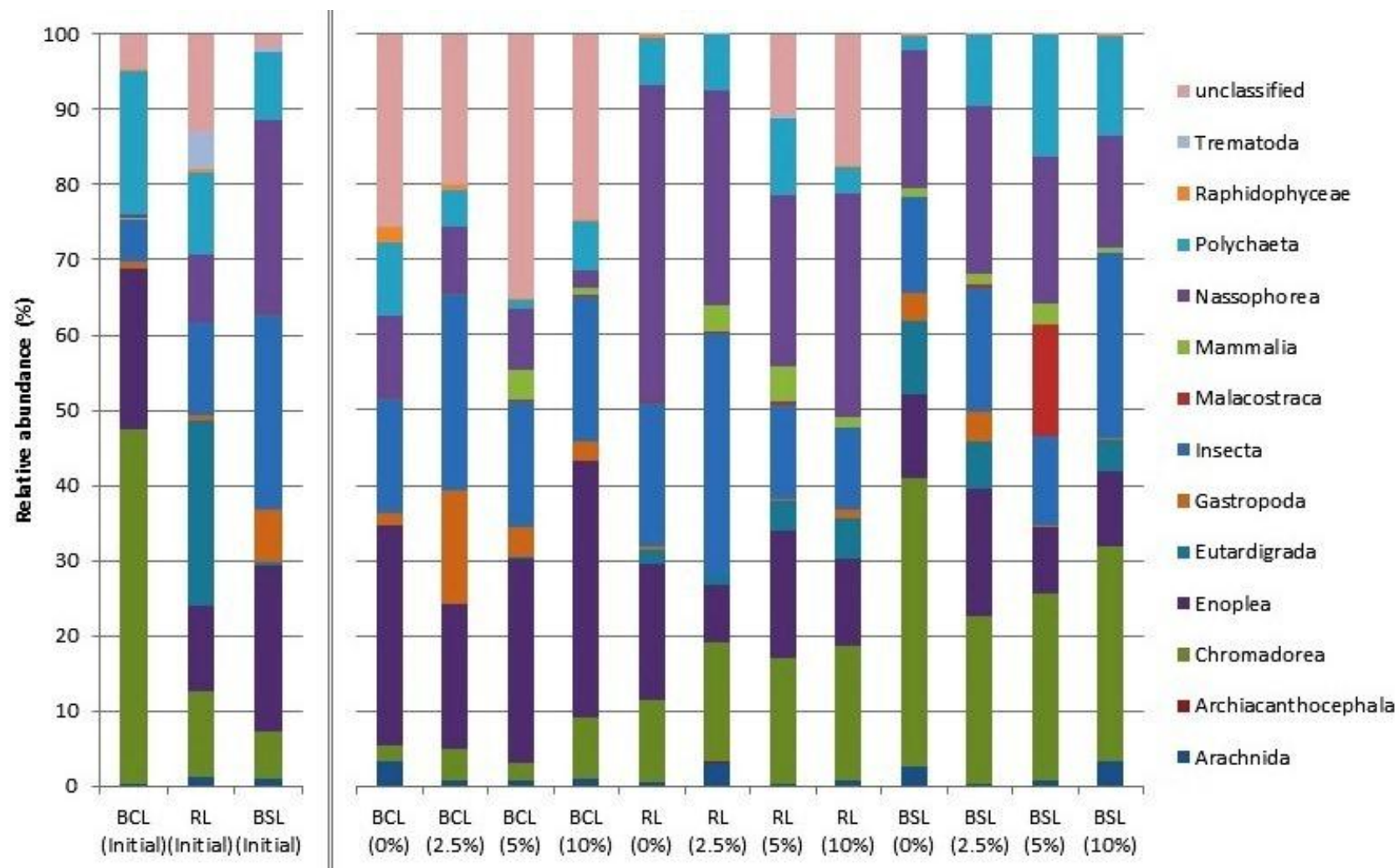


Figure 5.6: Abundance of metazoa and other eukarya groups at the class level in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) in the initial samples and biochar treatments.

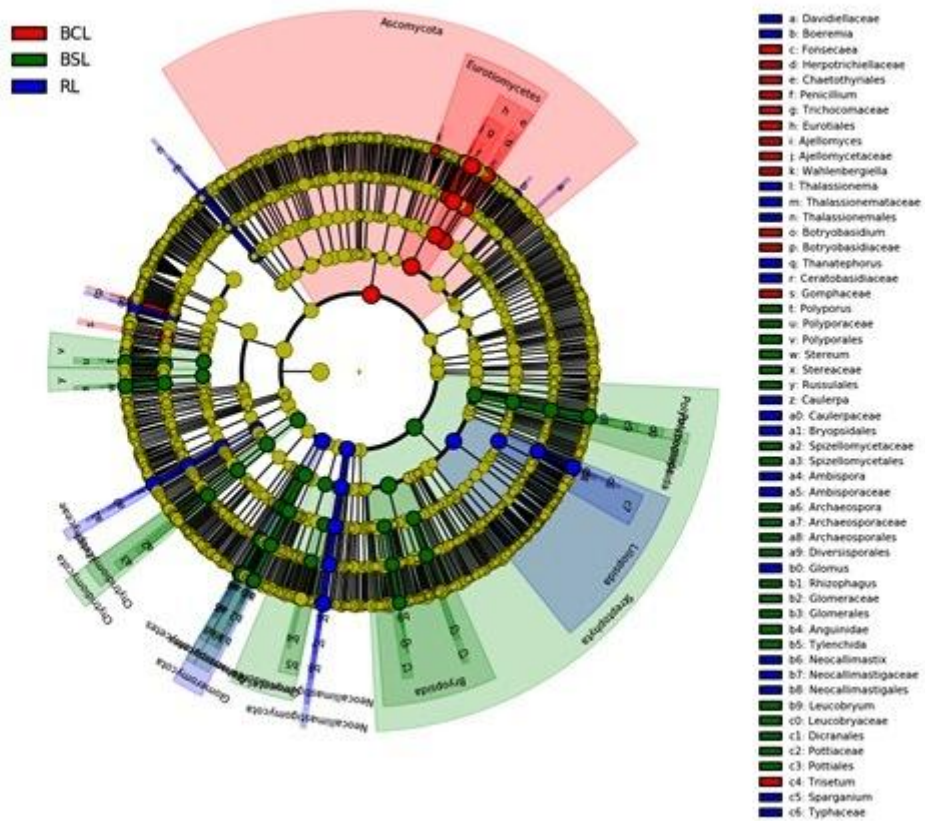


Figure 5.7: Phylogenetic distribution of the eukaryotic taxa (domain-genus level) with distinct relative abundance differences (LDA values of >3.5) in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) amended soils.

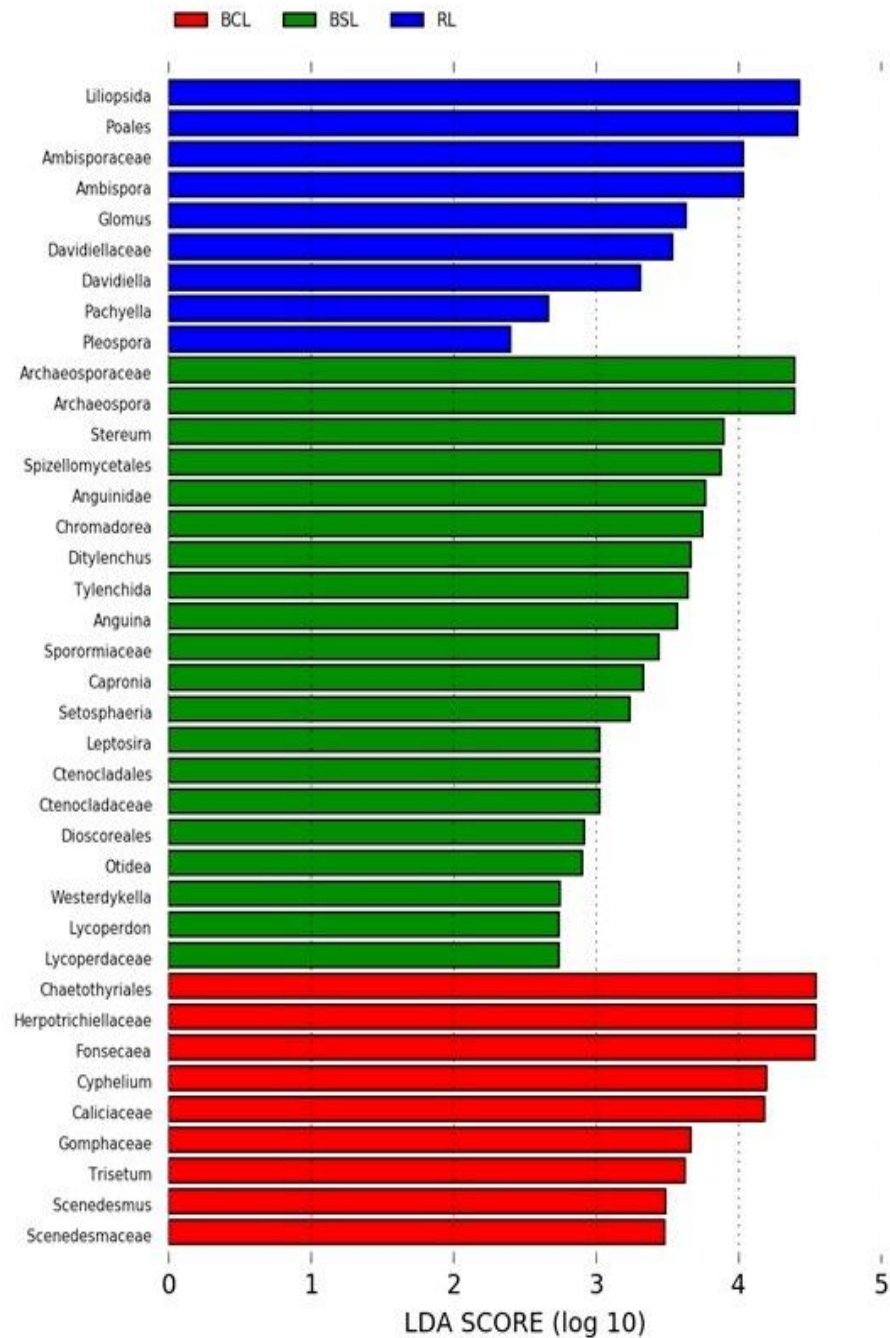


Figure 5.8: Linear discriminant analysis effect size (LEfSe) analysis showing the most significantly different eukaryotic taxa in terms of relative abundance in black clay loam (BCL), red loam (RL) and brown sandy loam (BSL) amended soils with LDA values of 3.5

4. Discussion

Biochar increases the pH and electric conductivity of soil (Kimetu et al., 2008; Chintala et al., 2014). Abujabhah et al. (2016b) indicated that biochar addition in non-fertilized soils raised pH values as loading rates increased in the RL and BSL soils that were investigated here. Biochar application also increased the total carbon in proportion to the increasing application of charcoal in all topsoils. The increases in soil pH and carbon after biochar addition provide conditions that can promote soil microbial abundances (Lehmann et al., 2011). Previous work on these soils (Abujabhah et al., 2016b) also indicated that different application rates of biochar impacts on soil moisture content, EC, NH_4/NO_3 ratios, total N, in a 10 month experiment, depending on the soil type. The degree of impact on microbial abundance by biochar is thus affected by a range of factors in addition to simply extra carbon accumulation (Gomez et al., 2014). The biochar carbon input also affects soil nutrient content and the relative impact could depend on initial soil C; overall this would explain the variety of biochar effects observed in different soils. Carbon content nevertheless is highly influential on soil microbial activity and growth (Alden et al., 2001; Yoshitake et al., 2007). Therefore, organic application not only affects soil chemical and physical properties, it also enhances nutrient cycling, both indirectly and directly (Ros et al., 2006). Since biochar is a very stable form of carbon, resistant to microbial degradation, available carbon utilised by soil microorganisms most likely is derived from different existing soil C sources already present or generated in the soil subsequent to biochar addition. The lighter topsoils were more affected by the addition of biochar than the heavier clay loam topsoil which indicate that the biochar impact is related to the changes in the physical properties like soil texture and structure following biochar application (Uzoma et al., 2011; Abujabhah et al., 2016b). Here we show that the 5 – 20 μm pores, which lie in the plant available water holding range, are

dominant and this might aid the lighter-textured soils more than the heavier-textured soils. It has been widely stated that biochar may increase the abundance of the microbial community in soil, which agrees with the results observed in this study especially in the BSL amended topsoil (O'Neill et al., 2009; Abujabhah et al., 2016a).

The multivariate results showed that biochar application significantly affects fungal and total eukaryotic communities but overall the diversity and richness remain relatively stable, possibly due to the short term nature of the modelling experiment, lack of nutrient input, and inherently patchy nature of localised eukaryotic communities, especially metazoa (Bahram et al., 2016). Eukaryote community structure alteration nevertheless could be due to enlargement of soil in macroporosity after biochar application (Hardie et al., 2014; Abujabhah et al., 2016a), for example greater abundance of *Polychaeta* and *Gastropoda* in the BSL and BCL topsoils, respectively. The results showed an increase in the *Glomeromycota* abundance especially in the lighter RL and BSL topsoils, possibly due to the enhancement of fungal colonisation which increase hyphae growth giving plants greater access to available P (Mickan et al., 2016). Different wild volunteer weedy vegetation grew during the experiment, most likely from the original soil based seed banks, and this would provide sites for the growth and survival of AM fungi. However, the differences in the AM were observed as a result of increasing biochar additions as compared to the control, occurring most especially in the RL and BSL soils. Hammer et al. (2014) stated that arbuscular mycorrhiza (AM) hyphae are able to access biochar microsites that are too small for most plant roots to enter ($<10\ \mu\text{m}$), and therefore increase P uptake from biochar. The dominant pore sizes in our biochar are within this size class ($5 - 20\ \mu\text{m}$) which retains water in the plant available range. The ability of AM fungi and Ectomycorrhiza affiliated to *Ascomycota*, *Basidiomycota* and *Zygomycota* to access biochar pores and hyphae colonisation

extension in or on the biochar surface potentially promotes plant growth and facilitates nutrient uptake. Despite the higher abundance of soil fauna in the initial samples, biochar additions seemed to increase the abundance of soil nematodes and protozoa in the lighter topsoil (BSL), this might be due to the increased bacterial and fungal abundance, which are considered a prey for soil fauna (Hallmann et al., 1999; Akhtar and Malik, 2000), in BSL amended soils. The enhancement of soil porosity after biochar application can be expected to stimulate the growth and colonisation of fungal hyphae and provide more suitable habitats for soil micro-mesofauna which may in turn affect the microbial community structure, nutrient transformations and overall soil health and fertility.

5. Conclusion

In summary, the eukaryotic community structure of the natural soils as assessed in the ‘initial’ soil samples showed they were noticeably different in structure to the soil placed in pots and incubated outside for 10-months. Despite this a measurable impact of biochar loading on soil communities, in particular the *Glomeromycota* (arbuscular mycorrhiza) in lighter-textured topsoils was observed. The arbuscular mycorrhiza was most likely hosted on the wild weedy vegetation which grew in the pots during the 10-month experiment. However, stronger soil microbiological changes were observed due to soil mixing, potting and incubation of unamended soils. The limited impact of biochar loading rates may also be in part due to the short incubation-time and the lack of added nutrients, other than those present in the biochar product. Patchiness is also a significant challenge for analytical purposes. To overcome the disturbance and timing issues a longer-term field experiment would be required with a larger number of replicates. Our work supports the need for further studies on the longer-term

impacts of biochar on eukaryotic community composition in conjunction with model experiments to examine the specific physical, chemical and biological variables in different soils.

Chapter 6

General discussion and conclusion

1. Overview

Since the transition from primitive bio-fuels (wood, charcoal, animal power) to fossil fuels (coal, oil and gas) as primary energy resources, environmental concerns have progressively increased on the impacts of increased anthropogenic CO₂ production on the Earth's atmosphere and climate. Amongst a vast range of technologies and processes that seek to reduce CO₂ levels in the atmosphere 'biochar' (charcoal) as a soil amendment has been proposed as a possible 'win-win' solution for sequestering more carbon in soils as well as increasing agronomic productivity (Spokas et al., 2012). The Amazonian *Terra Preta* soils provided the inspiration for the idea of adding charcoal to soil. Their long-term physical and chemical fertility is considered as a model for sustainable soil management and long-term C sequestration (Glaser and Birk, 2012). Terra Petra soils have a high fertility associated with long term "slash and burn" agricultural practices which have cause the accumulation of large amounts of burnt plant residues, including much charcoal (Mishra and Ramakrishnan, 1983; German, 2003). This accumulated carbon is believed to enhance soil properties and agronomic productivity. Despite the generally positive impacts of biochar amendment on soil properties and microbial activity (Atkinson et al., 2010; Lehmann et al., 2011), the impacts are quite varied and the exact reasons for this remain unclear. Based on the interaction between biochars from different feedstocks and pyrolysis temperatures with different application rates and soil types, considerable variation in response has been noted (Spokas and Reicosky, 2009; Van Zwieten et al., 2010), thus it would seem that responses depend on

an interaction between biochar and soil properties and the downstream influence this has on the soil microbial community.

2. Biochar characteristics and production

Wood and charcoal historically provided almost all fuel energies prior to the discovery of fossil fuels. Nevertheless, wood and green wastes are increasingly being used as renewable energy sources where biochar has emerged as a significant by-product (Spokas et al., 2012). The potential environmental benefits of biochar being used as a soil C sequestration method raised the focus on techniques that are used during production and methods and benefits of soil amendment. Therefore, an understanding of the quality of biochar produced from different feedstocks and under different conditions is required to enable selection of desirable biochars for soil applications (Lehmann and Joseph, 2009; Novak et al., 2009b). The feedstocks used and pyrolysis temperature produce biochar with different properties. Straw biochars have a high ash content and available nutrients compared to the wood biochars (Kloss et al. (2012). Most biochars are limited as a source of nutrients; however, biochar produced at high temperature has significantly more surface area compared to low temperature biochar which increases potential sorption capacity and nutrient retention in soils. In particular biochar with higher surface area might be more beneficial in sandy soils compared to heavier textured soils. High aromatic and stable biochars are crucial in C sequestration in soils which is a featured strategy in climate change mitigation. Kloss et al. (2012) stated that due to the variety of materials used to produce biochar, there is a potential risk of toxicity in relation to the feedstocks and temperature used for biochar production. Schimmelpfennig and Glaser (2012) suggested that different criteria can be used to choose a

suitable biochar for soil amendments. Their observations gave rise to analytical guideline values with threshold variables of O/C ratio <0.4 , H/C ratio <0.6 , black carbon $>15\%$ C, polycyclic aromatic hydrocarbons lower than soil background values and a surface area $>100 \text{ m}^2 \text{ g}^{-1}$. These variables could be used to assess the stability of biochar against degradation and to identify a suitable biochar for soil amendment and environmental management (Schimmelpfennig and Glaser, 2012). Furthermore, due to the different feedstock materials and to avoid any negative impacts of biochar, simple tests are needed to identify any toxic elements (Rogovska et al., 2012; Schimmelpfennig and Glaser, 2012). To ensure biochar can be safely applied to soils, further studies are required to chemically characterise the environmental effects and create a dataset for biochars due to the variety of feedstocks used in pyrolysis processes (Busch et al., 2012).

On the other hand, there is a debate about the expense of using biochar in agriculture and the benefits regarding crop productivity and climate change management. Much of the literature over the past decade has presented promising agricultural and environmental benefits of using biochar to improve soil fertility and mitigate climate change (Guo et al., 2016). However, there is a gap between the research and the actual application in relation to the cost and benefits of biochar amendments. Also, most of the research studies are conducted in developed countries with short term application for research interests, while fewer studies are conducted in developing countries for a long term field application (Zhang et al., 2016b). As a result, the lack of compiled information on a global scale limits our understanding of biochar as a commercial product and the potential agricultural and environmental profits that might be achieved to cover the cost of biochar amendment in soil. Life cycle assessments conducted by Roberts et al. (2009) to estimate the energy, climate change impacts and the economics of biochar systems that utilised corn stover residues, yard waste, and switchgrass

energy crops as feedstocks showed that the viability of biochar relied on the costs of feedstock production, pyrolysis, and the value of C offsets. The results also indicated that the net energy of switchgrass was the greatest in the system (4899 MJ t^{-1} dry feedstock) while the net greenhouse gas (GHG) emissions for both corn stover and yard waste are reduced by 864 and 885 kg CO₂ equivalent (CO₂e) emissions per tonne dry feedstock, respectively. The feedstock transportation cost seemed to be a barrier for the biochar-pyrolysis systems profit; however, biochar might be financially applicable and beneficial as a climate change mitigation strategy and waste biomass distribution system (Roberts et al., 2009). Galinato et al. (2011) estimated the economic value of C sequestration and soil properties in biochar amended soils, the study suggested that it might be profitable to use biochar as a soil amendment under the conditions of existing C offset markets and low price biochar. Under such conditions, biochar has the potential to increase C sequestration over a long time period, and profit the environment (Galinato et al., 2011).

Wrobel-Tobiszewska et al. (2015) evaluated biochar production from eucalypt plantations residue wood under Tasmanian conditions, the study concluded that biochar has a potential to deliver financial benefits to the forestry industry. The production costs and financial gains from savings in standard forestry procedures and biochar sale can be adjusted to fit local conditions and the total benefit depends on the final product distribution and biochar price (Wrobel-Tobiszewska et al., 2015).

3. Biochar and soil fertility

Soil fertility is related to many aspects, including biotic and abiotic reactions and their interactions in an array of complex and dynamic processes. Biochar application may affect

soil parameters due to its specific properties and the changes that occur in soil after biochar addition, which may involve a range of soil physical and chemical properties as well as microbial activity (Atkinson et al., 2010; Lehmann et al., 2011). The changes in one or more of these parameters in soil after biochar amendment may indirectly affect other aspects in relation to soil fertility which denotes the complexity of the biotic and abiotic interactions and activities in soil.

When added at sufficient levels biochar seems likely to change the soil, but the degree and benefits/detractions from these changes appears to be partly based on the specifications of the biochar applied. Biochar can provide high micro-porosity and surface area which can a potential habitat for microorganisms in soil. Also their capacity to increase CEC enhances the retention of nutrient cations and their availability in the soil for microbes and plant uptake (Atkinson et al., 2010). The application of biochar could also improve irrigation management and water infiltration and enhance fertiliser treatment response in soil. Asai et al. (2009) investigate the effect of biochar on physical properties and rice green yields. Their experiments, conducted within upland conditions in ten sites at application rates from 0-16 t/h combined with different N and P fertilizer application rates, showed an improvement in the hydraulic conductivity and increased rice yields in sites with low P content and also higher responses in fertilised biochar treatments than fertiliser alone. Improving hydraulic conductivity and other physical characteristics in soil provides suitable conditions for the chemical interactions and microbial activity. Furthermore, because biochar has high resistance to the microbial degradation; the impact of biochar addition in soil could persist for a long time (Asai et al., 2009; Lehmann et al., 2009). The use of biochar as a soil amendment could be more beneficial in soils that have poor physio-chemical characteristics, such as sandy soils. An experiment conducted by Basso et al. (2013) suggested that biochar addition

increased water retention in soil by 23% compared to the control. The result also showed that bulk density of the control soil increased during the incubation time of the experiment by almost 3%, while bulk density of biochar-treated soils was 9% less than the control and constantly stable during incubation time. Biochar contribution and impact on soil physical properties such as soil stability and aggregation, water management, porosity and surface area indicate that understanding biochar functions and effects in soil would assist the use of particular biochars to suit specific agricultural soils to gain the maximum benefits from using biochar as a soil amendment (Sohi et al., 2010).

The implications of biochar addition on soil chemical properties are complicated and unpredictable because specific chemical properties of each biochar are affected by the pyrolysis conditions and feedstock used to produce the biochar (Unger et al., 2011; Kloss et al., 2012). Unger et al. (2011) conducted an incubation experiment to determine whether biochar produced under different reactions from different feedstocks would differentiate the influence of biochar on soil chemical properties. In this study, selected parameters were measured included total nitrogen, total organic carbon, ammonium nitrogen ($\text{NH}_4\text{-N}$) and nitrate nitrogen ($\text{NO}_3\text{-N}$), the results suggested that the reaction conditions and organic materials used to produced biochar will affect specific chemical properties which therefore influence soil parameters in the amended soils. It has been stated that biochar may increase pH in soil which influence nutrients availability in soil (Fowles, 2007). However, the impact of biochar on soil pH is dependent on the pH of the biochar itself and the liming capacity which varies between different biochars (Kookana et al., 2011; Lehmann et al., 2011).

The impact of biochar on biotic processes and related microbes has been discussed recently by many researchers; however there remains a limited understanding regarding the interaction between biochar amended soils and the normal vs heightened or changes in micro

biological processes (Lehmann et al., 2011). Biochar contains highly stable forms of vitrified carbon which potentially increases the C/N ratio and affects the microbial activity and nutrient cycling in soil (Nguyen et al., 2008; Kookana et al., 2011). Improvement of soil physical and chemical properties by biochar amendment may enhance microbial activity as it is likely to be source of nutrients and a suitable habitat for soil microorganisms. Investigating the effect of biochar addition on microbial biomass and activity, Kolb et al. (2009) added biochar to four different soils (Mollisol, Alfisol, Entisol, and Spodosol) at five application rates from 0 to 0.1 kg/kg⁻¹ soil. Their results showed a significant increase in both microbial biomass and activity with increasing application rates. The same patterns of biochar impact were observed on microbial biomass, microbial activity and nutrient availability in all four soils but the microbial response to biochar varied depending on the differences in nutrient availability in each soil. Solaiman et al. (2010) found that phosphorus solubility increased in the presence of biochar due to an increase in mycorrhizal colonisation, however, the results showed a decrease in microbial respiration with increasing application rates in biochar amended soil (Thies and Rillig, 2009; Solaiman et al., 2010). In contrast, other studies have shown an increase in total respiration and respiratory rate, and a reduction in mycorrhizal colonization after biochar application (Treseder, 2004; Steinbeiss et al., 2009). The differences in biochars, application rates and type of soils are likely to have contributed to the range of effects of biochar on microbial communities.

The effect of biochar on soil fertility can be positive or negative depending on the quality of the biochar and application rates (Spokas et al., 2012), hence there are many aspects which still need to be investigated. Furthermore, changes in soil nutrients often occur over a long period of time and most of the studies reported in the literature were conducted over a relatively short time period. Hence longer term experiments with observations of plant

response are required for a comprehensive determination of the impact of biochar on soil fertility.

4. Research benefits and key findings

This thesis investigated the impact of biochar addition on physical and chemical properties and interactions with the microbial community. Biochar was applied in an apple orchard field site and compared to compost application under conventional agricultural practices for 3.5 years. In addition a controlled a pot-based experiment was conducted to evaluate the impact of biochar loading rates and in three very different topsoil types. Several soil chemical and biological tests were conducted to evaluate the changes in biochar amended soils and the potential benefits of biochar application. The basic goal was to understand how green waste-derived biochars affected soil properties – primarily chemistry, biology and the actual community structure. The studies undertaken in the apple orchard site complement other studies with an agronomic emphasis including assessment of tree growth and an assessment of soil physical properties. The pot experiment on the other hand was a shorter term evaluation to try to highlight biochar impacts in a lower input system in which soil management inputs like irrigation and fertilisation were eliminated and instead the key variables were distinct topsoil types.

4.1. Chemical and physical properties

The findings from Chapter 2 address a field trial based comparison between Acacia green waste derived biochar and compost applied to a commercial apple orchard. The results

indicated that there was no significant impact on any of the measured nutrient anions and cations in either biochar or compost treatments with an active nutrient management regime in the orchard. It was concluded that this was most likely a result of the management practices of this particular commercial orchard where high levels of fertilisers were regularly applied, leading to swamping of the biochar impacts on soil fertility (Eyles et al., 2015). As anticipated organic carbon was significantly increased ($p=0.009$) for biochar (23%) and even more so for compost treatments (55%). Surprisingly soil pH decreased in both biochar and compost treatments and this was attributed to the broadcasting of raw chicken manures and other fertilisers across the whole site.

Chapter 3, which was based on a 10-month curing of various soil-biochar mixtures in pots placed outside. This experiment showed that biochar also increases soil carbon levels but this time it showed an increase in soil pH, though this varied with soil type. The increase of total carbon was associated with an increase C/N ratio due to the very high C/N ratio of the biochar. In general, adding carbon to a soil increases labile soil carbon decomposition and N utilisation by microorganisms (Lehmann et al., 2011; Nelson et al., 2011). The pot experiment described in Chapter 3 showed a reduction of the available N and organic carbon in soil after biochar application for all three soil types. High biochar loading rates appear to also influence nitrification and the function and activity of microbial community in lighter soils. These changes occurred after biochar additions and might potentially affect microbial activity, at least for long-term biochar amendment. The stability of C in biochar makes it an ideal strategy for C sequestration. While there is some available C in low temperature biochars for biodegradation, biochar C is more stable in soil than the C in other organic materials (Ippolito et al., 2012). Therefore, adding high level of carbon in the form of biochar seems to be a reliable way of keeping carbon in the soil for longer periods of time in order to

gain extended improvements of soil characteristics, C sequestration and climate change mitigation.

The pot experiment showed that biochar additions had a significant impact on NH_4 and NO_3 , total C and N, pH, EC and soil moisture content in both a soil type and loading dependent manner. In heavier and reactive soil, such as the clay loam - Black Vertosol, no significant impact was observed on the available P and K levels, nor the total exchangeable base cations (TEB) and CEC. However, in the other lighter soils, such as loam – Red Dermosol and sandy loam – Brown Kurosol, biochar addition had a significant effect on the exchangeable Al, Ca, Mg, Na levels and CEC. Thus, biochar addition to lighter sandy soil increases water holding capacity which increases the available water content in soil as well as improving nutrient availability and more generally soil physio-chemical properties in lighter soils (Sohi et al., 2010; Basso et al., 2013).

Soil pH increased progressively after biochar application with increasing loading rates in the pot experiment (Chapter 3), while pH decreased in the field study. This increase in soil pH with increasing biochar application rates was expected due to the alkalinity of the biochar ($\text{pH} > 9$) used in this study (Kimetu et al., 2008; Chintala et al., 2014). However, a decrease in soil pH after the addition of biochar and compost in the field site (Chapter 2) may be due to the lower pH (6.4) and hence liming value of the biochar used in the field site. Furthermore, increasing the organic matter following the addition of biochar or compost combined with the impact of fertiliser additions may also decrease soil pH due to the microbial activity and organic acids released during organic matter decomposition. As different biochars were used in the field and pot experiments, the effect of biochar on soil pH is dependent on the pH of biochar itself and the liming value resulting from different feedstocks and pyrolysis conditions used for biochar production (Kookana et al., 2011; Lehmann et al., 2011). Biochar

with high pH and liming capacity might be used in acid soils to increase pH and thus improve nutrient availability.

4.2. Microbial structure and activity

The results of this study illustrate a limited impact of biochar on microbial biomass, composition and activity at least in the short term. Results from the field site experiment (chapter 2) indicated that the application of biochar and compost can subtly influence soil characteristics leading to changes in bacterial and fungal community structure more than three years after the original application of the amendments. The changes in eukaryote community structure could be associated with enhancement in macro-porosity and bioturbation in the soil after biochar amendment (Hardie et al., 2014). The alteration in the fungal community structure was the most evident while a lower impact was observed for the overall bacterial community structure compared to untreated soils. The archaeal community, mainly involved in nitrification were not affected by the amendments. In the pot study, biochar addition had a limited effect on the microbial biomass; however, the interaction between biochar and different soils significantly affected the potential nitrification especially in RL and BSL soils. There were significant differences in C substrate utilisation among biochar treatments in the three different soils. The opposite effects in substrate utilization rates as biochar levels increased in the BCL and BSL soils compared with the RL soil indicate that biochar may influence the function and activity of microbial communities. Furthermore, biochar addition had a greater impact on the bacterial diversity in RL and BSL than the BCL soil. The relative abundance of nitrifying bacteria increased with increasing biochar rate, especially AOB in BCL soil. However, the relative abundance of *Nitrospira* and

NOB was greater than AOB in all biochar amended soils. It has been widely documented that biochar reduces N₂O emissions in agricultural soils (Zhang et al., 2010; Liu et al., 2012b; Zhang et al., 2016a), this may be due to the adsorption of N by biochar reducing the available N to denitrifying and/or nitrifying bacteria and archaea in soils (Singh et al., 2010b). Although no N₂O flux measurements were conducted in this study, the enhancement of NOB abundance over AOB and the observed effect of biochar on potential nitrification in the amended soils may possibly reduce N₂O emissions due to reduction of available N to nitrifying microbes (Cayuela et al., 2013) in combination with suppression of the denitrification function (Li et al., 2016). Changes in nitrifier community composition has the potential to change the nitrification patterns, yet the mechanisms of biochar impact on AOB and NOB are still uncertain and require more attention (He et al., 2016a). Although nitrogen fixing bacteria respond differently to biochar amendments in different soils, the relative abundance of selected groups of nitrogen fixing bacteria (*Bradyrhizobium*, *Azospirillum*, *Frankia* and *Herbaspirillum*) were increased after biochar additions. The impact of biochar on biological nitrogen fixation might be as a result of nutrient enhancement in the amended soils, especially K availability (Mia et al., 2014), which may lead to increases in nitrogenase activity in the biochar amended soils (Quilliam, 2013). Therefore, further investigation is required to explore the mechanisms of biochar impact on nitrogen fixation for long term application. The significant increase in abundance of methanotrophs with increased biochar application rates especially in BCL soil, suggest that the changes in methanotrophs community composition and methanogens or methylotrophic activities induced by the changes in physicochemical properties after biochar application will possibly affect methane production in amended soils (Liu et al., 2011; Feng et al., 2012; Yu et al., 2013).

The results of the studies reported in this thesis also point to largely subtle changes in the eukaryotic community composition when comparing biochar amended soils with unamended controls. The impact of biochar additions on the fungal communities and total eukaryotes was most discernible in the lighter BSL soil compared to the BCL and RL soils. In this respect *Glycomycota* appears to be slightly promoted in BSL soils, possibly due to altered soil porosity and chemistry. The eukaryotic community structure of the soils represented in the initial samples in the pot study were noticeably different to the experimentally manipulated samples, possibly due to the mixing of soil and biochar during preparation. The modest impact of biochar loading on soil communities was possibly due to the short time incubation and the lack of nutrient availability in the biochar amended soils. Longer-term trials with grass cover or other natural carbon sources would seem to be a logical extension of this work.

5. Future research and conclusion

It is clear that biochar has potential for use as a soil amendment with benefits for greater environmental management and crop productivity. However, there are some aspects that still need to be considered to maximise agricultural, economic and environmental benefits of biochar and to overcome the high cost of biochar production and application.

- Recent focus has been on short term experiments which investigate the impact of biochar on selected soil chemical and physical properties, thus more field application research is needed to evaluate the long term impact of biochar application and the interactions between biochars derived from different feedstocks under different pyrolysis conditions in different soils.

- In addition to the use of biochar as a soil amendment to improve fertility and crop productivity, biochar needs to be recognised as an effective strategy to mitigate climate change and C sequestration as well as other potential products of the thermochemical pyrolysis of biomass materials. Therefore, more research is needed in developing countries where the costs and profits of biochar production and application are adjusted to be economically suitable for different needs and requirements. As a result, sustainable land management through biochar utilization may promote poverty reduction through increased soil fertility and productivity.
- Biochar use and application needs to be globalised to suit most environments and economics, therefore, more research needs to be conducted in developing countries where the costs and profits of biochar production and application are adjusted to be economically suitable for different needs and requirements. As a result, sustainable land management through biochar utilization promotes poverty reduction by increasing soil fertility and productivity.
- As there are a variety of organic materials used in biochar production, ranging from plant residues to waste products, toxicity tests and contamination studies are required to avoid any negative effects of biochar application to soil. Also creating a dataset for biochar classification would help in choosing suitable biochar for soil amendment based on chemical characteristics. Such a classification tool has been described by Camps-Arbestain et al. (2015), where biochar properties are classified based on a set of physicochemical properties to meet soil-crop needs (International Biochar Initiative, http://www.biochar-international.org/classification_tool). At present, properties that are classified include carbon storage value, fertilizer value (P, K, S, and Mg only), liming value and particle size distribution. The systematic use of this categorisation

with experiments and practices described above will aid in the realisation of effective biochar application.

- Finally, more attention is needed to understand the impact of biochar amendment on soil microbial dynamics, specifically in relation to nutrient cycling and biodegradation. The effect of biochar on soil microorganisms is still not fully clarified, and the interaction between different biochars and soils may affect microbial communities differently depending on the original soil status and the changes that occur after biochar application.

In conclusion, this thesis has explored several aspects of biochar application in soil. The significance of this work was the evaluation of the impact of biochar amendment in a conventional field site and pot studies with a short term and relatively longer term application as well as the interaction between different loading rates of biochar and soil types. This work has shown promising results regarding biochar application and has also allowed a better understanding of the interaction between biochar and different soil systems and the impacts on soil physicochemical properties and microbial community. The findings of this work demonstrated the potential benefits of biochar amendment but have also shown that future research is needed to explore more aspects of the impact of biochar on soil fertility and crop productivity, as well as the potential benefits for sustainable agriculture and climate change management in biochar amended soils.

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